

# Short Report: Association of IL-15 with Peripheral and Hepatic Insulin Sensitivity in Healthy Middle-Aged Men

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## Abstract

Rodent study suggests that interleukin (IL)-15 administration enhances insulin sensitivity. Although it is well known that circulating levels of typical inflammatory markers (C-reactive protein [CRP] and IL-6) are positively associated with homeostasis model assessment-insulin resistance (HOMA-IR), there are no studies investigating the associations of other inflammatory markers including IL-15 with peripheral/hepatic insulin sensitivity in humans. The current study aimed to examine the relationship between the levels of adipokines or inflammatory cytokines and insulin sensitivity in 8 healthy middle-aged men. Circulating levels of 10 insulin sensitizing adipokines or inflammatory cytokines (total adiponectin [APN], high molecular weight adiponectin [HMW-APN], IL-4, IL-5, IL-6, IL-8, IL-15, interferon [IFN]- $\gamma$ , tumor necrosis factor [TNF]- $\alpha$ , and TNF- $\beta$ ) were measured. A stable-labeled frequently sampled intravenous glucose tolerance test was performed to assess peripheral ( $S_I^{2*}$ ) and hepatic ( $hS_I^2$ ) insulin sensitivity estimated by 2-compartment minimal model. The levels of 3 inflammatory cytokines (IL-4, IL-6, and IL-15) were significantly and inversely correlated with either  $S_I^{2*}$  and  $hS_I^2$ . The association between IL-15 and either  $S_I^{2*}$  or  $hS_I^2$  was significant even after adjusting for age and percent body fat ( $p < 0.01$ ). The current study showed a possible inverse association between serum IL-15 level and peripheral/hepatic insulin sensitivity in healthy middle-aged males, independent of percent body fat; this association in humans warrants further study.

## Keywords

**Inflammatory Cytokine; Interleukin; Adipokine; Insulin Sensitivity**

### 1. Introduction

Low-grade chronic systemic inflammation plays an important role in the pathogenesis of obesity and type 2 diabetes [1] [2]. Inflammatory cytokines such as interleukin (IL)-6 and tumor necrosis factor (TNF)- $\alpha$  induce insulin resistance and suppress molecules involved in insulin signaling [2]. Intravenous administration of TNF- $\alpha$  impairs peripheral insulin sensitivity assessed by euglycemic hyperinsulinemic clamp in healthy humans [3] [4]. Consistently, epidemiological studies have shown that higher levels of serum IL-6 and C-reactive protein (CRP) were associated with the increased risk of type 2 diabetes [5] [6].

Studies have reported a positive relationship between the circulating levels of representative inflammatory markers (e.g., CRP, IL-6, and TNF- $\alpha$ ) and homeostasis model assessment-insulin resistance (HOMA-IR) [7] [8]. Recently, other cytokines such as IL-4, IL-8, and IL-15 have emerged as risk factors for adverse health. Inflammatory cytokines such as IL-4, IL-8, and IL-15 are involved in the process of atherogenesis [9]-[11]. For instance, an epidemiological study showed that serum IL-15 concentration in hypertension patients with severe organ damage (88.7 pg/mL) was significantly higher than that in those with no organ damage (55.2 pg/mL) [12]. On the other hand, rodent studies suggest that IL-15 has beneficial effects in the regulation of glucose metabolism. It has been demonstrated that IL-15 administration increased insulin sensitivity in obese mice [13]. There is, however, no information on the associations between the levels of inflammatory cytokines such as IL-4, IL-8, and IL-15 and insulin sensitivity in humans.

A stable-labeled 2-compartment minimal model can provide indices of peripheral insulin sensitivity ( $S_I^{2*}$ ), as well as glucose effectiveness ( $S_G^{2*}$ ) (*i.e.*, ability of glucose to stimulate its own uptake), insulin secretion, and endogenous glucose production (EGP) in patients and healthy individuals [14]-[16]. Additionally, this model enabled us to assess hepatic insulin sensitivity (*i.e.*, the ability of insulin to inhibit EGP,  $hS_I^2$ ) and hepatic glucose effectiveness (*i.e.*, the ability of glucose to inhibit EGP,  $hS_G^2$ ) [17]. Therefore, we examined the relationships between the circulating levels of 10 adipokines or cytokines including IL-15 and peripheral/hepatic insulin sensitivity indices ( $S_I^{2*}$  and  $hS_I^2$ ) assessed by a stable-labeled 2-compartment minimal model in healthy middle-aged men.

### 2. Materials and Methods

**Subjects:** The study subjects were 8 healthy middle-aged men with normal glucose tolerance. We included healthy participants with a wide range of insulin sensitivity to examine the associations between serum cytokines and insulin sensitivity indices. Of these 8 subjects, 4 men were sedentary, defined as not engaged in any habitual exercise for at least 2 years, and 4 were runners, defined as those who run  $79 \pm 27$  km/week. None of the subjects were taking any medications or supplements at the time of study enrollment. Before beginning the study, the nature, purpose, and the risks of the study were explained to all subjects, and written informed consent was obtained. The protocol was approved by the local ethical committee of the Jichi Medical University and was conducted in accordance with the Helsinki Declaration.

**Anthropometric indices, oral glucose tolerance test, and stable-labeled frequently sampled intravenous glucose tolerance test:** Body mass index (BMI) was determined by dividing body weight in kilograms by the square of height in meters. Each subject's percent body fat was measured by hydrostatic weighing and was estimated based on the hydrostatic density with a correction for the residual lung volume [18]. An oral glucose tolerance test (OGTT; 75 g glucose) was performed at least 7 days prior to the frequently sampled intravenous glucose tolerance tests (FSIGTTs) to confirm normal glucose tolerance in all subjects. Stable-labeled FSIGTTs were performed, as previously described [14]-[16]. Briefly, after overnight fasting, glucose (300 mg/kg body weight) (isotopically labeled with [6, 6- $^2$ H $_2$ ] glucose [Aldrich, Milwaukee, WI]) was administered in the contralateral antecubital vein within 1 min. Regular insulin (Humalin; Shionogi, Osaka, Japan) was infused (20 mU/kg) into the antecubital vein from 20 to 25 min after the glucose bolus. Blood samples for glucose and insulin were frequently obtained up to 180 min. On the day before undergoing the FSIGTT, all subjects were provided with

an evening meal consisting of  $\geq 140$  g carbohydrate,  $\geq 30$  g fat, and  $\geq 33$  g protein.

Deuterated glucose was analyzed as a penta-acetate derivative, and  $S_I^{2*}$  and  $S_G^{2*}$  specific to glucose uptake, and EGP were estimated by a 2-compartment minimal model [14]–[16]. Hepatic insulin sensitivity (*i.e.*, the ability of insulin to enhance glucose suppression of EGP,  $hS_I^2$ ) and hepatic glucose effectiveness (*i.e.*, the ability of glucose to inhibit EGP,  $hS_G^2$ ) were also analyzed as previously described [17]. To assess endogenous insulin secretion, insulin area above the basal level between 0 and 20 min after the administration of glucose was calculated using the conventional trapezoid rule. The glucose disappearance constant ( $K_G$ ) was calculated as the slope of the least squares regression line related to the natural logarithm of the glucose concentration to the time from samples drawn between 10 and 19 min. The HOMA-IR was calculated as the product of the fasting serum insulin concentration ( $\mu\text{U/mL}$ ) and fasting plasma glucose concentration ( $\text{mg/dL}$ ) divided by 405 [19].

**Measurement of serum cytokine:** Fasting levels of serum total-APN and HMW-APN were measured by enzyme-linked immunosorbent assay (ELISA) (Human adiponectin ELISA kit, Human HMW-adiponectin ELISA kit, Otsuka Pharmaceutical Company, Tokushima, Japan). Both intra- and inter-assay coefficients of variation for total-APN and HMW-APN were  $< 15\%$ . Fasting levels of serum IL-4, IL-5, IL-6, IL-8, IL-15, IFN- $\gamma$ , TNF- $\alpha$ , and TNF- $\beta$  were measured by multiplex sandwich ELISA kit (Q-Plex™ Human Cytokine, Quansys Bioscience Inc., Utah, USA), according to the manufacturer's instructions. Chemiluminescence was quantified with a Lumino-Image Analyzer LAS-3000 System (Fujifilm Inc., Tokyo). Lower detectable limits for IL-4, IL-6, IL-8, IL-15, and TNF- $\alpha$  were 0.39, 0.93, 0.24, 0.60, and 1.37 pg/mL, respectively. A good agreement between signal intensity and cytokine level was observed within the assay range ( $r^2 \geq 0.99$ ). Intra- and inter-assay coefficients of variation were 10.6% and 17.4% for IL-4, respectively; 7.6% and 18.2% for IL-5, respectively; 6.5% and 11.7% for IL-6, respectively; 4.1% and 20.5% for IL-8, respectively; 5.4% and 14.7% for IL-15, respectively; 8.9% and 15.9% for IFN- $\gamma$ , respectively; 6.1% and 18.8% for TNF- $\alpha$ , respectively; and 4.3% and 14.4% for TNF- $\beta$ , respectively. All cytokines were measured 3 times, in duplicate, and the average of the 3 measurements was used for data analyses. Free fatty acid (FFA) levels were measured by standard method.

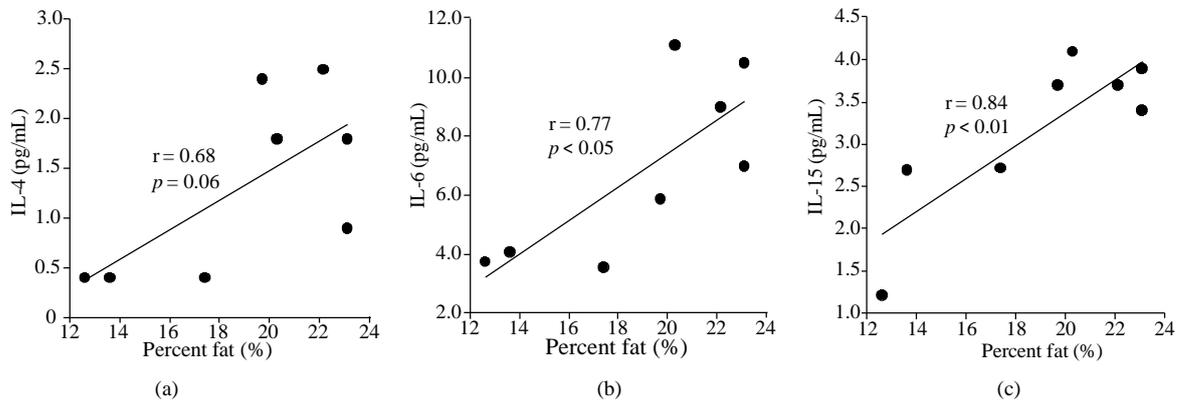
**Statistical analysis:** Values are shown as the mean  $\pm$  SD. The significance of the correlation between variables was assessed by the Pearson correlation coefficient. Multiple regression analyses with adjustment for age and percent body fat were performed to examine if cytokine levels (independent variables) could be associated with insulin sensitivity indices (dependent variables), independent of percent body fat. A  $p$  value of  $< 0.05$  was considered statistically significant. All analyses were performed using SAS software (version 9.3 for Windows, SAS Institute, Cary, NC, USA).

### 3. Results

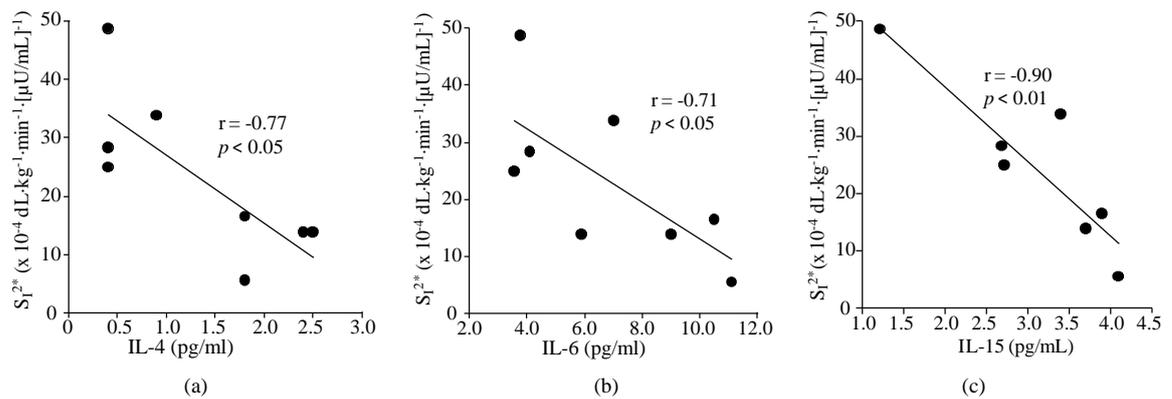
General characteristics of the study subjects are shown in **Table 1**. The percent body fat tended to be positively correlated with IL-4 ( $r = 0.68$ ,  $p = 0.06$ ), and it was significantly and positively correlated with IL-6 and IL-15 (**Figure 1**). Likewise, BMI was positively correlated with the levels of IL-4 and IL-6 (IL-4:  $r = 0.84$ ,  $p < 0.01$ ; IL-6:  $r = 0.80$ ,  $p < 0.05$ ; IL-15:  $r = 0.65$ ,  $p = 0.08$ ). Insignificant correlations were observed between either percent body fat or BMI and the other 7 cytokines (total-APN, HMW-APN, IL-5, IL-8, IFN- $\gamma$ , TNF- $\alpha$ , and TNF- $\beta$ ) (data not shown). Three cytokines (IL-4, IL-6, and IL-15) were inversely correlated with  $S_I^{2*}$  (**Figure 2**), while correlations between these cytokines and insulin secretion, insulin-independent glucose uptake ( $S_G^{2*}$ ), or basal EGP were not statistically significant (data not shown). Additionally, the 3 cytokines (IL-4, IL-6, and IL-15) showed significant inverse correlations with  $hS_I^2$  (**Figure 3**), whereas among the 3 cytokines but only IL-6 was significantly and inversely correlated with  $hS_G^2$  ( $r = -0.73$ ,  $p < 0.05$ ).

When the analyses were performed separately for sedentary men ( $n = 4$ ) and runners ( $n = 4$ ), the correlation between percent body fat and IL-15 became non-significant in sedentary men ( $r = -0.71$ ,  $p = 0.29$ ) and runners ( $r = 0.86$ ,  $p = 0.14$ ). On the other hand, the relationship between IL-15 and  $S_I^{2*}$  tended to be significant in sedentary men ( $r = -0.93$ ,  $p = 0.07$ ) and remained significant in runners ( $r = -0.995$ ,  $p < 0.01$ ). Additionally, the correlation between IL-15 and  $hS_I^2$  remained significant in sedentary men ( $r = -0.97$ ,  $p < 0.05$ ) and runners ( $r = -0.993$ ,  $p < 0.01$ ).

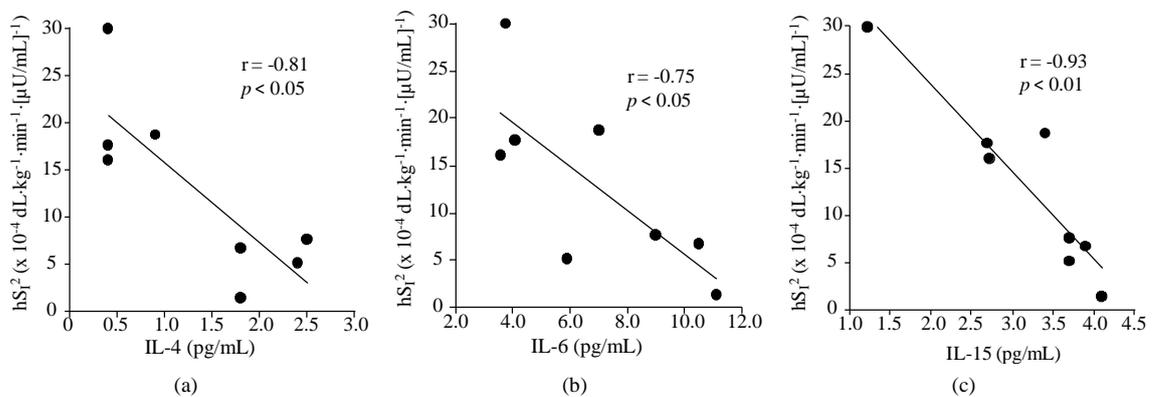
In the multiple regression analysis using data from all subjects ( $n = 8$ ), the associations between either IL-4 or IL-6 and  $S_I^{2*}$  became insignificant, whereas that between IL-15 and  $S_I^{2*}$  remained statistically significant after the adjustment for age and percent body fat (**Table 2**). The inverse relationship between either IL-4 or IL-15 and  $hS_I^2$  were also significant even after adjusting for age and percent body fat (**Table 2**). Additionally, among



**Figure 1.** Correlations between percent body fat and the levels of 3 inflammatory cytokines (IL-4, IL-6, and IL-15) in healthy middle-aged individuals.



**Figure 2.** Relationships between the levels of 3 inflammatory cytokines (IL-4, IL-6, and IL-15) and  $S_1^2$  in healthy middle-aged men.



**Figure 3.** Associations between the levels of 3 inflammatory cytokines (IL-4, IL-6, and IL-15) and  $hS_1^2$  in healthy middle-aged males.

cytokines that were measured, only IL-6 was significantly and positively correlated with HOMA-IR ( $r = 0.71$ ,  $p < 0.05$ ), but this association became insignificant after adjusting for age and percent body fat.

#### 4. Discussion

In the current study, we observed inverse associations between the circulating level of IL-15 and either peri-

**Table 1.** General characteristics of the study subjects.

	Men (n = 8)
Age (years)	48.3 ± 4.9
BMI (kg/m <sup>2</sup> )	22.3 ± 0.1
Percent fat (%)	19.0 ± 4.1
IL-4 (pg/mL)	1.33 ± 0.91
IL-5 (pg/mL)	2.67 ± 0.78
IL-6 (pg/mL)	6.86 ± 3.05
IL-8 (pg/mL)	5.34 ± 1.04
IL-15 (pg/mL)	3.18 ± 0.95
IFN- $\gamma$ (pg/mL)	4.42 ± 0.85
TNF- $\alpha$ (pg/mL)	5.42 ± 1.19
TNF- $\beta$ (pg/mL)	1.30 ± 0.27
Total-APN ( $\mu$ g/mL)	5.2 ± 2.2
HMW-APN ( $\mu$ g/mL)	3.9 ± 1.0
Basal glucose (mg/dL)	97.8 ± 8.4
Basal insulin ( $\mu$ U/mL)	5.4 ± 2.5
HOMA-IR	1.3 ± 0.7
Basal FFA (mEQ/L)	0.31 ± 0.13
Postload glucose (mg/dL) <sup>a</sup>	108.3 ± 20.7
K <sub>G</sub> (% min <sup>-1</sup> )	1.87 ± 0.57
Insulin area 0 - 10 min ( $\mu$ U/mL·min)	231 ± 145
Insulin area 0 - 20 min ( $\mu$ U/mL·min)	332 ± 212
$S_i^{2*}$ ( $\times 10^4$ , min <sup>-1</sup> ·[ $\mu$ U/mL] <sup>-1</sup> ·dL·kg <sup>-1</sup> )	22.7 ± 14.0
$S_g^{2*}$ ( $\times 10^2$ , min <sup>-1</sup> ·dL·kg <sup>-1</sup> )	0.65 ± 0.11
Basal EGP (mg/kg/min)	1.70 ± 0.18
$hS_i^{2*}$ ( $\times 10^4$ , min <sup>-1</sup> ·[ $\mu$ U/mL] <sup>-1</sup> ·dL·kg <sup>-1</sup> )	12.93 ± 9.38
$hS_g^{2*}$ ( $\times 10^2$ , min <sup>-1</sup> ·dL·kg <sup>-1</sup> )	0.87 ± 0.25

Values are mean  $\pm$  SD. BMI, body mass index; IL, interleukin; IFN, interferon; TNF, tumor necrosis factor; APN, adiponectin; HMW-APN, high molecular weight-adiponectin; HOMA-IR, homeostasis model assessment-insulin resistance; FFA, free fatty acid; K<sub>G</sub>, glucose disappearance constant;  $S_i^{2*}$ , peripheral insulin sensitivity;  $S_g^{2*}$ , peripheral glucose effectiveness; EGP, endogenous glucose production;  $hS_i^{2*}$ , hepatic insulin sensitivity;  $hS_g^{2*}$ , hepatic glucose effectiveness. <sup>a</sup>Plasma glucose concentration in 2-h oral glucose tolerance test.

**Table 2.** Multiple regression analyses with 3 serum cytokines as predictors for peripheral/hepatic insulin sensitivity.

	$S_i^{2*}$		$hS_i^{2*}$	
	$\beta$	<i>p</i> value	$\beta$	<i>p</i> value
IL-4	-15.3	0.08	-10.8	<0.05
IL-6	-2.8	0.32	-1.9	0.30
IL-15	-21.0	<0.01	-13.4	<0.01

Adjusted for age and percent body fat. IL, interleukin;  $S_i^{2*}$ , peripheral insulin sensitivity;  $hS_i^{2*}$ , hepatic insulin sensitivity.

peripheral ( $S_i^{2*}$ ) or hepatic ( $hS_i^{2*}$ ) insulin sensitivity in healthy middle-aged men, independent of percent body fat. When data were analyzed in sedentary men (n = 4) as a more homogeneous group, a similar pattern of results

regarding these inverse relationships as those analyzed in all subjects ( $n = 8$ ) were obtained; this corroborates the current results. This is an unexpected finding, since IL-15 administration has been linked to higher insulin sensitivity in obese mice [13]. To our knowledge, there is no previous study that simultaneously assessed IL-15 and insulin sensitivity in humans.

There is a previous study showing that the serum IL-15 concentration in the exercise- and diet-induced weight-loss group was decreased by 33% in obese individuals [20]. In another study, the circulating level of IL-15 in the physical exercise intervention group decreased by 2.8% in older adults, although this decrease was not statistically significant [21]. In this previous study, physical exercise intervention significantly reduced the serum level IL-15 by 10% in older participants with a high baseline IL-15 [21]. Additionally, it is well known that insulin sensitivity is increased with moderate exercise training [16] [22], and the current results suggest that lower IL-15 could be associated with higher peripheral/hepatic insulin sensitivity. Our cross-sectional study is limited by the fact that we are not able to analyze the association between the longitudinal changes in IL-15 and insulin sensitivity before and after the intervention. Thus, we can only speculate that the decreased levels of circulating IL-15 may be associated with improved insulin sensitivity after diet and/or exercise program in humans.

The biological mechanisms underlying the association of IL-15 with insulin sensitivity are largely unclear, but there are a few possible explanations for the association. It has been shown that IL-15 induces nuclear factor- $\kappa$ B (NF- $\kappa$ B) activation in human neutrophils [23]. Since hepatic activation of NF- $\kappa$ B induces local and systemic insulin resistance [24], IL-15 might act directly on insulin-targeted organs such as skeletal muscle and liver to worsen insulin sensitivity, assuming that IL-15 induces NF- $\kappa$ B activation in insulin-sensitive organs. There is also a possibility that IL-15 exerts its adverse effect on insulin sensitivity through regulating other factors, such as FFA and IL-6. IL-15 stimulates lipolysis in primary pig adipocytes [25]. Prolonged increases in FFA, which is produced as a result of lipolysis (in company with glycerol), impair insulin action to promote glucose transport in skeletal muscle [26]. Thus, FFA might act as a mediator between IL-15 and peripheral insulin sensitivity. In this connection, higher IL-15 tended to be correlated with higher FFA in the current subjects ( $r = 0.53$ ,  $p = 0.17$ ). Additionally, a previous study showed that high IL-15 concentrations enhanced IL-6 production in cell culture [27]. Consistently, higher IL-15 was correlated with higher IL-6 in the current subjects ( $r = 0.80$ ,  $p < 0.05$ ). Since IL-6 induces insulin resistance [1] [2] [28], IL-6 could be a possible mediator involved in the mechanisms underlying the inverse association between IL-15 and insulin sensitivity.

On the other hand, the possibility that IL-4 plays a role in regulation of serum IL-15 level cannot be denied. A previous study demonstrated that IL-15 mRNA expression was induced in human blood-derived dendritic cells during culture in granulocyte-macrophage colony-stimulating factor and IL-4 [29]. In this previous study, the induction of IL-15 gene expression was followed by a moderate increase of IL-15 protein in the culture supernatants [29]. Also, higher IL-4 was associated with higher IL-15 in the present study ( $r = 0.76$ ,  $p < 0.05$ ).

Rodent study suggests that IL-15 takes part in reducing the mass of adipose tissue. IL-15 administration for 7 days decreased white adipose tissue by approximately 30% in adult rats [30]. Consistently, Nielsen *et al.* [31] showed an inverse association between plasma IL-15 and percent fat mass was also observed in humans. On the other hand, the current study observed a positive relationship between percent body fat and IL-15 concentration in healthy middle-aged men who are sedentary or runners. When analyzing with data from 4 sedentary men, the correlation between percent fat and IL-15 became non-significant, as described in the result section. Thus, we are not able to exclude the possibility that the significant correlation observed in all subjects ( $n = 8$ ) could be due to heterogeneity of the current participants (*i.e.*, sedentary men and runners). Further studies are needed to determine the association between IL-15 and body fat in rodents and humans.

This study had several limitations. First, sample size of this study was very small. We were unable to increase the number of subjects due to an insufficiency of serum samples to measure cytokines. Despite the small sample size, we were able to confirm previously reported association of IL-6 with either body fat or insulin sensitivity [7] [32] by using most precise methods to measure percent body fat, serum cytokine levels, and peripheral/hepatic insulin sensitivity. This confirmation validates current methods and results. However, it is evident that further research is needed to examine if similar relationships are observed in a larger sample of subjects with varying degree of obesity, since the current participants only include men with mild obesity (percent body fat of 24% at the most). Also, additional risk factors potentially affecting insulin sensitivity in healthy humans (*e.g.*, family history of lifestyle-related diseases, blood pressure, and lipid profiles) were not examined. Although we considered percent body fat to be the most important factor influencing insulin sensitivity, incomplete adjustment for other potential confounding variables might have influenced the results. Moreover, causal-

ity cannot be judged by this type of study. Longitudinal studies are highly warranted to investigate the association between the chronological changes in IL-15 and insulin sensitivity. Detailed mechanisms for associations between IL-15 and peripheral/hepatic insulin sensitivity remain to be elucidated.

## 5. Conclusion

The current study showed a possible inverse association between serum IL-15 level and peripheral/hepatic insulin sensitivity in healthy middle-aged men, independent of percent body fat; this association in humans warrants further study.

## Disclosure Statement

The authors declare that they have no conflict of interest.

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