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Changes in Plasma Adiponectin, IL-6, TNF-α and Free Fatty Acid Concentrations in Obese Japanese Men

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MS and DK designed and performed experiments. Authors MS and YS analyzed the data. Authors MS, DK and MS contributed to write the manuscript. Authors SI, NN, YI and JS contributed to Discussion. All authors read and approved the final manuscript.

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Short Communication

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ABSTRACT

The risk of developing metabolic syndrome, hypertension, type 2 diabetes, and hyperlipidemia increases with obesity, and an elevated visceral fat content has been associated with a higher incidence of metabolic risk factors. We investigated differences in IL-6 (Interleukin-6), TNF-α (tumor necrosis factor-α), adiponectin and FFA (free fatty acid) levels in obese and non-obese Japanese men. Five obese men (BMI: 32.4±4.9 kg/m^2) and five non-obese men (BMI: 23.2±2.9 kg/m^2) participated in this study.IL-6 levels were significantly higher in obese than in non-obese subjects, whereas no significant differences were observed in TNF-α, adiponectin, or FFA levels. These results suggested that IL-6 levels may be affected more by obesity in Japanese men than TNF-α and adiponectin levels, which may, in turn, influence the pathophysiology of obesity in Japanese individuals.

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ABBREVIATIONS

IL-6: interleukin-6; TNF-α: tumor necrosis factor-α; FFA: free fatty acid.

1. INTRODUCTION

The risk of developing metabolic syndrome, hypertension, type2 diabetes, and hyperlipidemia is known to increase with obesity, which activates the immune system, thereby affecting metabolic homeostasis [1]. Metabolic syndrome is not a single disease, it is a cluster of different conditions, and has been reported not only in the United States, but also in the remainder of the urbanized world [2]. Adipose tissue in obese individuals is filtrated by a large number of macrophages, resulting in systemic inflammation. Different subsets of macrophages are involved in obesity-induced adipose tissue inflammation: M1, the classically activated macrophage phenotype, and M2, the alternatively activated macrophage phenotype [3]. The inflammatory response associated with obesity changes the levels of circulating cytokines including IL-6 (Interleukin-6), TNF-α (tumor necrosis factor-α), and adiponectin. IL-6 and TNF-α are proinflammatory cytokines that are secreted from macrophages, T cells, and B cells, and activate the inflammatory system. Between 10 and 30% of IL-6 is secreted from adipose tissues [4]. IL-6 levels were previously shown to be significantly higher, while adiponectin levels were significantly lower in obese children and adolescents [5], and also correlated with insulin resistance and other cardiovascular factors. Inflammation has also been associated with inactivity-induced muscle atrophy after bed rest; the expression of IL-6 mRNA was increased following bed rest [6].

Adiponectin exerts protective actions against obesity-linked diseases such as atherosclerosis and insulin resistance by attenuating chronic inflammation [7]. A reduction in circulating adiponectin levels has been linked to the development of insulin resistance [8]. Adiponectin also exhibits a protective effect against hypertension through an endothelium-dependent mechanism [9], and this was shown to be suppressed in obese subjects [10].

Increases in visceral fat content have been associated with a higher incidence of metabolic risk factors in Japanese men [11]. Obesity is defined by a BMI of 25 kg/m^2 or above in Japan (The Examination Committee of Criteria for 'Obesity Disease' in Japan, 2002). The Japanese criteria for waist circumference cut-offs are also lower than in other non-Asian countries such as Europe and in USA [12]. The genetic background of Africans and East Asians makes them more susceptible to diabetes than Caucasians [13], and insulin sensitivity was previously reported to be higher in Japanese subjects than in Caucasians [14]. Genetic backgrounds are also known to influence the secretion of cytokines; IL-6 levels were found to be higher in South Asian women than in European women [15]. A higher visceral fat content, together with a greater number of adipose-tissue macrophages, has been shown to produce more proinflammatory cytokines such as TNF-α and IL-6 and less adiponectin [16].

In the present study, we investigated differences in IL-6, TNF-α, adiponectin, and FFA levels in young obese Japanese men using the Japanese criteria for obesity (BMI>25.0 kg/m^2).We hypothesized that IL-6, TNF-α, and FFA concentrations may be higher while adiponectin concentrations may be lower in obese men than in non-obese men in Japan.

2. METHODS

Five obese men (age: 30±5 yrs; BMI (body mass index): 32.4±4.9 kg/m^2; body mass: 92.3±15.5 kg; height: 169±27 cm; waist circumference: 103±17 cm; body fat percentage: 31.2±5.2%, mean±SE) and five non-obese men (age: 24±3yrs; BMI: 23.2±2.9 kg/m^2; body mass: 66.6±10.4 kg; height: 169±27 cm; waist circumference: 76±12 cm; body fat percentage: 19.6±2.9%) participated in this study, as shown in Table 1. Fat mass and fat percentages were measured by a bioelectrical impedance analysis (Tanita Co., Japan). Obesity was defined as a BMI of 25 kg/m^2 or higher (The Examination Committee of Criteria for 'Obesity Disease' in Japan); therefore, obese men who matched this criterion were selected. A previous one-year study showed no significant alterations in body mass, and subjects followed their usual sleep-wake schedules during the experiment. A medical examination confirmed that none of the subjects had cardiovascular abnormalities or inflammatory diseases. Subjects were asked to

refrain from the consumption of alcohol and beverages containing caffeine, smoking, taking medication, and performing exercise for 12 hours before any test session. Subjects were also asked to continue with their usual routine before each part of the study; however, the composition of their diet was not controlled. All subjects gave written informed consent before the experiment, which was approved by the Ethics Committee, Aichi Medical University, and adhered to the guidelines advocated by Chronobiology International [17].

Experiments were performed in Aichi prefecture (Lat. 35º10' N, Long. 136º57.9'E), Japan between December and January in 2010/2011. The average ambient temperature during the experimental period was 3.3±0.4ºC. Each subject rested for 30 min in a climatic chamber set at 26ºC and 50% relative humidity, and blood samples were collected by venipuncture. Samples were collected between 1330 hours and 1600 hours. Blood samples were centrifuged, and the serum was separated and stored at -80ºC until the analyses for serum concentrations of TNF-α, IL-6, adiponectin, and FFA. The analytical methods used were as follows: TNF-α was assayed by a commercially available enzyme-linked immunosorbent assay (ELISA; R&D Systems Inc., Minneapolis, USA); IL-6 by a commercially available chemiluminescence enzyme immunoassay (CLEIA; FUJIREBIO Inc., Tokyo, Japan); adiponectin by a latex particle-enhanced turbidimetric immunoassay (automated analyzer, JCA-BM12, JEOL Ltd., Tokyo, Japan); and FFA by an enzyme method (automated analyzer, Bio Majesty, JCA-BM2250, JEOL Ltd., Tokyo, Japan).

Pearson product-moment correlation coefficients were calculated between BMI and TNF-α, IL-6, adiponectin, and FFA. Comparisons between all variables in obese and non-obese subjects were performed using the Mann Whitney test. Significance was set at $p<0.05$. All data were expressed as mean±SE.

3. RESULTS

Table1 shows the main anthropometric characteristics of the non-obese and obese subjects. Weight, BMI, waist circumstance, and hip circumstance were significantly higher in obese than in non-obese subjects. A positive correlation was noted between IL-6 and BMI (R=0.733; p=0.016), and IL-6 concentrations were significantly higher in obese than in non-obese subjects (3.46±0.96 pg/mL in obese subjects; 1.16±0.17 pg/mL in non-obese subjects, p=0.016) (Fig. 1A). TNF-α and BMI were positively correlated (R=0.706; p=0.023), whereas no significant differences were observed in TNF-α concentrations between obese and non-obese subjects (1.42±0.30 pg/mL in obese subjects; 0.94±0.12 pg/mL in non-obese subjects, p=0.151) (Fig. 1B). Neither adiponectin nor FFA concentrations correlated with BMI (R=-0.479, p=0.161 for adiponectin; R=-0.122, p=0.774 for FFA), and no significant differences were observed in adiponectin (7.66±1.91 μg/mL in obese subjects; 12.0±3.54 μg/mL in non-obese subjects, p=0.310) or FFA concentrations (399±15.0 μEq/L in obese subjects; 401±67.3 μEq/L in non-obese subjects, p=0.786)between obese and non-obese subjects (Figs.1C and D).

Table 1. Main anthropometric characteristics in non-obese and obese subjects

	Non-obese	obese	p-value
Age (years)	24±3	30±5	0.421
Weight (kg)	66.6±10.4	92.3±15.5	0.032
Height (cm)	169±27	169±27	0.690
BMI (kg/m^2)	23.2±2.9	32.4±4.9	0.016
Fat (%)	19.6±2.9	31.2±5.2	0.056
Waist circumference (cm)	76±12	103±17	0.008
Hip circumference (cm)	96±15	113±18	0.008

p-values were calculated using Mann Whitney test

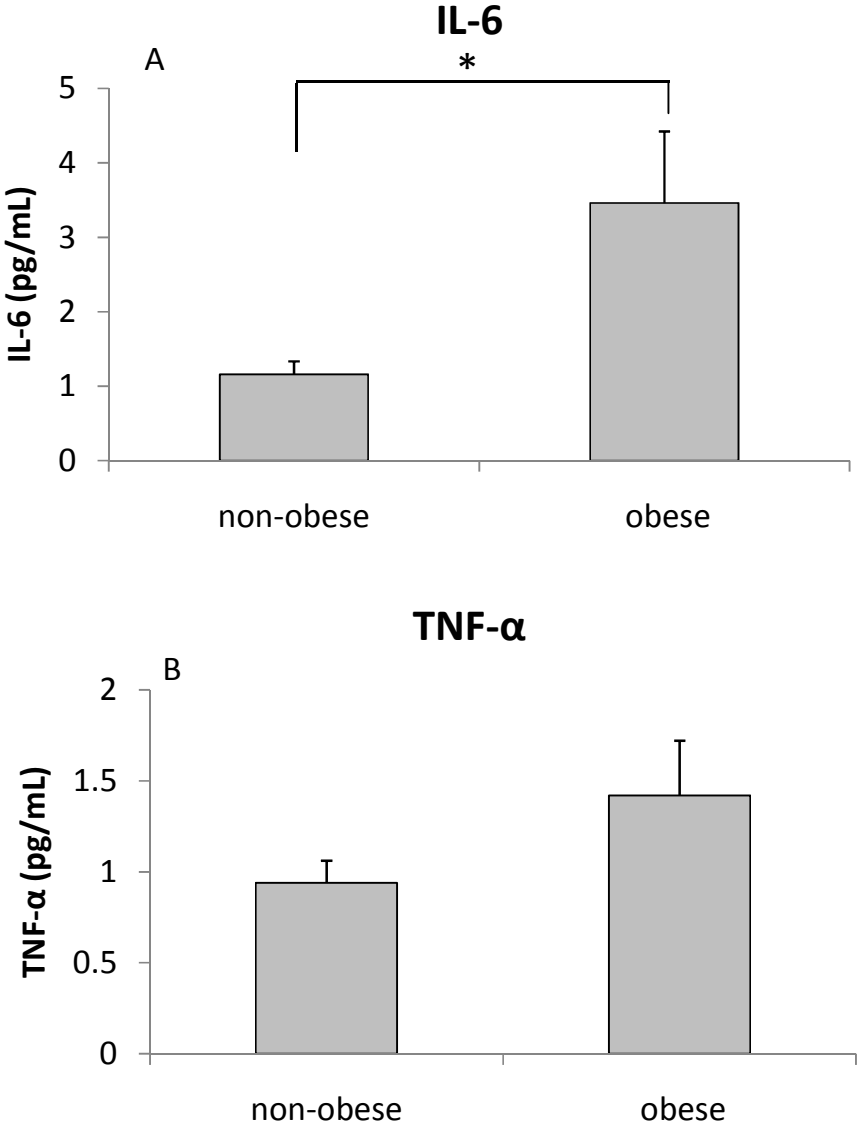
4. DISCUSSION

We examined differences in IL-6, TNF-α, adiponectin, and FFA levels between obese (BMI, 32.4±4.9 kg/m^2) and non-obese (BMI: 23.2±2.9 kg/m^2) young Japanese men. We found that: 1) IL-6 was significantly higher in obese than in non-obese subjects; and 2) no significant differences were observed in TNF-α, adiponectin, or FFA levels between obese and non-obese subjects. IL-6 is a pro-inflammatory cytokine that influences the pathophysiology of obesity. IL-6 has been correlated with visceral adiposity in obese patients [18,19]. IL-6 levels were previously shown to be significantly decreased by a 2.3±0.2 kg reduction in the total fat mass due to dermolipectomy [20], suggesting that fat mass is a key factor determining IL-6 levels. In our study, fat percentages were 31.2±5.2% in obese and 19.6±2.9% in non-obese subjects, while the calculated fat masses were 28.8±0.81 kg in obese and 13.05±0.3 kg in non-obese

subjects. The increased fat mass in obese subjects may have enhanced the secretion of IL-6.

No significant differences were observed in TNF-α levels between obese and non-obese subjects, whereas a correlation was found between TNF-α and BMI (R=0.706; p=0.023). Previous studies showed that TNF-α levels were elevated with obesity [21]. The obese state of BMI>25 kg/m^2 in the present study was lower than that of BMI>30 kg/m^2 in other studies, which may have affected our results; therefore, TNF-α levels may be higher in obese individuals with BMI larger than 30kg/m^2.TNF-αlevels were previously shown to be decreased by prolonged and moderate intensity exercise in young obese Japanese women [22].

We found no significant differences in adiponectin levels between obese and non-obese subjects. Adiponectin prevents the development of arteriosclerosis and insulin resistance. It is also inhibited by TNF-α, and a negative correlation has been reported between TNF-α and adiponectin levels [23]. Decreases in adiponectin levels have been associated with increases in IL-6 and TNF-α levels in epicardial adipose tissue [24]. These findings implicate the lack of a significant difference in TNF-α levels for adiponectin levels being similar between obese and non-obese subjects in the present study.



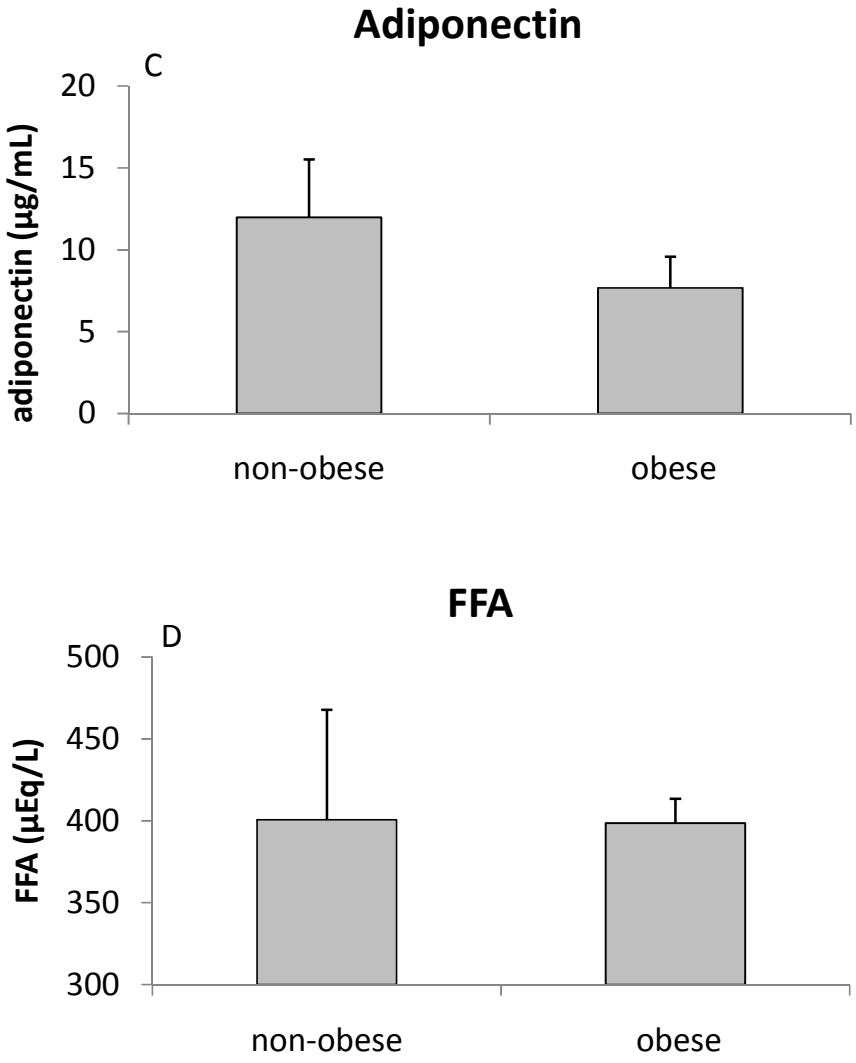


Fig. 1. IL-6 (A), TNF-α(B), adiponectin (C) and FFA (free fat acid)(D) levels between non-obese and obese subjects. Results are presented as the mean±SE. *, a significant difference at p<0.05wascalculated by Mann Whitney test. The definition of obesity was BMI≥25 kg/m^2 and non-obesity was BMI<25 kg/m^2

No significant differences were observed in FFA between obese and non-obese subjects in our present study. Lee and Jensen [25] found no correlation between plasma FFA levels and BMI in Koreans or Caucasians. The timing of FFA release from adipose tissues has to be controlled because excess levels of lipids in the circulation may lead to lipotoxicity and promote cardiovascular disorders [26]. Therefore, FFA concentrations may not be influenced by obesity.

We demonstrated that IL-6 was significantly higher in obese Japanese men with BMI>25 kg/m^2 without elevations in TNF-α, adiponectin, or FFA levels. Our results indicated that IL-6 plays a more important role in the pathophysiology of obese Japanese men than the other cytokines examined because the percentage of individuals with BMI <30 kg/m^2 among the obese population in Japan (24.8% men and 28.0% women) was higher than those with BMI >30 kg/m^2 (1.9% men and 3.2% women) [27]. These results suggest that IL-6 is influenced by obesity at BMI greater than 25 kg/m^2 in Japanese men. These results also

suggest the possible involvement of IL-6 in the development of metabolic syndrome and its complications in Japanese individuals.

5. LIMITATIONS

The present study has several limitations, in particular the small number of subjects (n=10). A larger number of subjects will enable differences between obese and non-obese subjects to be more easily identified. Furthermore, the methodology of laboratory examinations was different for IL-6 and TNF-α due to technical reasons; CLEIA for IL-6 (standard detection level: 0.2-4.0 pg/mL) and ELISA for TNF-α (standard detection level:0.6-2.8 pg/mL). In addition, inflammatory diseases were confirmed in our subjects through a medical examination only, and not with any laboratory parameter (i.e. erythrocyte sedimentation rate and leukocyte count).

6. CONCLUSION

It is concluded that IL-6 plays an important role in the pathophysiology of obese Japanese men, and the possible involvement of IL-6 in the development of metabolic syndrome and its complications in Japanese individuals.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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