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# A Study of Fecal Calprotectin in Obese Children and Adults

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**Background:** Obesity is a complex, medical condition causally contributing to many chronic diseases and a number of efforts have been made to find the associated markers for novel prevention and treatment of obesity. Our study was to evaluate the relationship between gut immune response and obesity and overweight with use of fecal calprotectin (FC) both in adult and children groups.

**Methods:** Fecal samples were obtained from 74 subjects: 14 non-obese and overweight children (PN), 13 obese and overweight children (PO), 20 non-obese and overweight adults (AN), and 27 obese and overweight adults (AO). FC was measured using a commercial Legend Max quantitative enzyme-linked immunosorbent assay (BioLegend). Mann-Whitney *U*-test was used for statistical analysis.

**Results:** Median FC concentration was 7.9 μg/g (range, 1.9–28.9 μg/g) for PN, 5.0 μg/g (range, 2.6–29.6 μg/g) for PO, 9.5 μg/g (range, 0.8–28.9 μg/g) for AN, and 10.0 μg/g (range, 1.6–25.6 μg/g) for AO, respectively. In both adults and children age groups, the FC showed no statistically significant difference between AO and AN or PO and PN. However, FC showed statistically significant difference ($P<0.05$) between AO and PO while not significant between AN and PN.

**Conclusion:** FC level in AO was significantly higher than that in PO, suggestive of different pathophysiologic mechanism between children obesity and adults obesity.

**Key words:** Obesity, Leukocyte L1 antigen complex, Immunity, Mucosal

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## INTRODUCTION

Obesity contribute substantially to mortality and are major risk factors for many chronic diseases including diabetes, vascular disease, and cancer.[1] Obesity and overweight in both children and adults continues to rise globally.[2] A number of efforts have been made to find the associated markers in the quest for novel prevention and treatment of obesity. The studied markers include adipocytic markers (adipokines: leptin, adiponectin)[3], hepatic or pancreatic markers (insulin-like growth factor 1, C-reactive protein [CRP], insulin)[4], gastrointestinal markers (ghrelin, cholecystokinin).[5]

Attention to intestinal immunity has been grown in the pathogenesis of various diseases. This includes the observation that the gut inflammatory profile in patients with type 1 diabetes is distinctive compared with healthy control subjects and gut inflammatory disease controls.[6] Additional reports showed that the colon mucosa from the patients with rheumatoid arthritis (RA) expressed unique three peptides involved in citrullination, an inflammation-dependent process, which could contribute to the onset of RA.[7] It is well known that neutrophils and monocytes in gut release calprotectin which is one of the key role players in gut immunity.[8,9] Our study was to evaluate the relationship between gut immune response and obesity and overweight with use of fecal calprotectin (FC) both in adult and children groups.

**Table 1.** Demographic characteristics of the study population

| Variable | PN (n=14) | PO (n=13) | *P* | AN (n=20) | AO (n=27) | *P* |
|---|---|---|---|---|---|---|
| Age (yr) | 9.6±3.2 (3.1–14.5) | 10.2±3.5 (6.36–17.5) | 0.522 | 47.6±11.8 (22.7–63.6) | 46.1±11.1 (25.7–62.2) | 0.692 |
| Sex (male:female) | 6:8 | 8:5 | 0.351 | 1:19 | 12:15 | 0.002 |
| Height (cm) | 140.7±22.6 | 143.8±16.6 | 0.687 | 159.6±7.4 | 166.0±11.8 | 0.037 |
| Height (Z-score) | 0.5±0.7 | 0.4±1.1 | 0.914 | NA | NA | NA |
| Weight (kg) | 32.6±12.6 | 51.0±16.1 | 0.003 | 50.5±7.4 | 73.2±9.9 | <0.001 |
| Weight (Z-score) | –0.6±0.8 | 1.6±0.9 | <0.001 | NA | NA | NA |
| BMI (kg/m$^2$) | 15.9±2.4 | 23.9±3.0 | <0.001 | 19.7±1.2 | 26.6±2.2 | <0.001 |
| BMI (Z-score) | –1.1±1.1 | 2.0±0.8 | <0.001 | NA | NA | NA |

Values are presented as mean±SD (range) or mean±SD.
PN, non-obese and overweight children; PO, obese and overweight children; AN, non-obese and overweight adults; AO, obese and overweight adults; NA, not applicable; BMI, body mass index; SD, standard deviation.

## METHODS

### Subjects

In the present study, fecal samples were obtained from 74 subjects: 14 non-obese and overweight children (PN), 13 obese and overweight children (PO), 20 non-obese and overweight adults (AN), and 27 obese and overweight adults (AO) (Table 1). Written informed consent was obtained from the subjects or their guardians. Diagnosis was based on the World Health Organization criteria in Asians as those with a BMI ≥23 and ≥25 kg/m$^2$ for overweight and obese adult[10] and criteria of Korea Centers for Disease Control Prevention as those with BMI ≥85th and ≥95th percentiles for overweight and obese children.[11] The subjects in the study met the exclusion criteria , based on a study of factors affecting levels of FC[12], which are as follows: no history of colorectal or systemic inflammatory condition, proton pump inhibitor use, regular (≥4 doses per week) use of nonsteroidal anti-inflammatory drugs, smoking or alcohol. The study was approved by the Ethical Committee of Cheju Halla General Hospital (No. CHHIRB-2015-L06-01).

### Fecal calprotectin

Subjects provided a single fecal sample for calprotectin measurement. Fecal samples were frozen on receipt at –80°C for further analysis. Before quantitative analysis, the frozen fecal samples were kept at room temperature (RT) for 30 minutes. After thawing, a single 100-mg aliquot was suspended in 1 mL of fecal extraction buffer (0.1 M Tris buffered saline with Tween 20, pH 8.0 [MB Cell, Los Angeles, CA, USA] with 0.5% bovine serum albumin, 0.15 M NaCl, 10 mM $CaCl_2$), and homogenized for 5 minutes with an vortex mixer (Scientific Industries, Bohemia, NY, USA). The homogenates were centrifuged for 15 minutes at 10,000 *g* at RT. The upper portion of the supernatants were pipette off, frozen, and stored at –80°C until quantitation by enzyme-linked immunosorbent assay.

FC was measured using a commercial Legend Max quantitative assay (BioLegend, San Diego, CA, USA). In details, the frozen fecal extracts were thawed and diluted 1:10 in the assay buffer. Standards and diluted samples (50 μL) were added to the plates which were covered and incubated at RT for 1 hour on a plate while shaking at 200 rpm. The wells were washed four times with wash buffer and 100 μL of human MRP8/14 detection antibody solution was added to each well, the plate covered, and incubated at RT for 30 minutes while shaking. Thereafter the wells were washed five times, 100 μL of substrate solution was added. The reaction was stopped by adding 100 μL of stop solution and the absorbance was read at both 450 nm and 570 nm using PowerWave XS2 Microplate spectrophotometer (BioTek, Winooski, VT, USA). The absorbance at 570 nm was subtracted from the absorbance at 450 nm. Calprotectin was expressed in micrograms per gram of fecal sample.

### Statistics

Data were analyzed using the GraphPad Prism (GraphPad Software, La Jolla, CA, USA) statistical software package. Calprotectin values were presented as mean±standard deviation, median and ranges. A Student *t*-test was used to evaluate the significance of the mean differences of demographic characteristics between PN, PO

**Table 2.** Descriptive statistics of fecal calprotectin levels in four groups

| Group | Mean ± SD (μg/g) | Median (range, μg/g) |
|---|---|---|
| PN (n=14) | 11.9±9.6 | 7.9 (1.9–28.9) |
| PO (n=13) | 7.8±7.8 | 5.0 (2.6–29.6) |
| AN (n=20) | 11.6±9.7 | 9.5 (0.8–28.9) |
| AO (n=27) | 12.7±7.5 | 10.0 (1.6–25.6) |

SD, standard deviation; PN, non-obese and overweight children; PO, obese and overweight children; AN, non-obese and overweight adults; AO, obese and overweight adults.

and AN, AO groups. Mann-Whitney *U*-tests were performed to compare FC between PN, PO, AN, and AO groups. The correlation between FC levels and BMI in children and/or adult was analyzed with Pearson or Spearman correlation analyses. All significant values were two-sided and $P<0.05$ was considered to indicate a statistically significant difference.

## RESULTS

Median FC concentration was 7.9 μg/g (range, 1.9–28.9 μg/g) for PN, 5.0 μg/g (range, 2.6–29.6 μg/g) for PO, 9.5 μg/g (range, 0.8–28.9 μg/g) for AN, and 10.0 μg/g (range, 1.6–25.6 μg/g) for AO, respectively (Table 2). In both adult and children age groups, the FC showed no statistically significant difference between AO and AN or PO and PN. However, in detail, FC was higher in AO than in AN while in children, the FC was higher in PN than in PO though statistically not significant. FC showed statistically significant difference between AO and PO while FC showed no significant difference between AN and PN (Fig. 1). The correlation analyses showed no significant correlation between FC and BMI in child and/or adult groups.

## DISCUSSION

Growing research suggests that obesity is not a simple phenomenon form improper diet and lifestyle, but a multi-factorial medical condition beyond individuals' control.[13] Among the factors, growing evidence suggests inflammatory links with obesity. Tumor necrosis factor (TNF)-α expression in the adipose tissue was reported to be increased in obese humans several decades ago.[14] Recent mice experiments showed that only TNF-α among multiple inflammatory cytokines was upregulated by high fat diets specifically in the

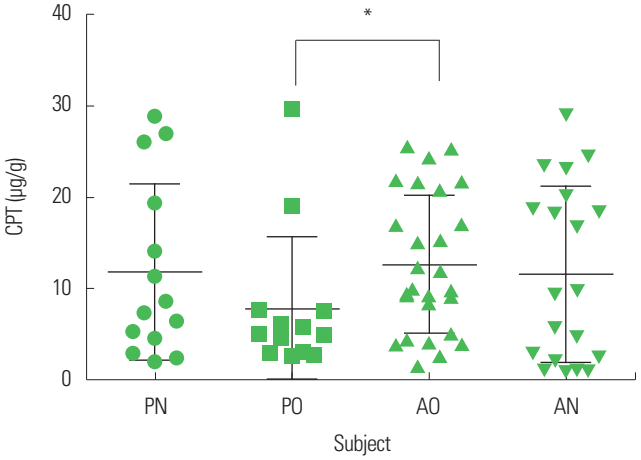


**Figure 1.** Scatter plots of fecal calprotectin (FC) levels in four groups. Comparison of FC levels between non-obese and overweight children (PN), obese and overweight children (PO), obese and overweight adults (AO), and non-obese and overweight adults (AN). The data were presented as mean±standard error of mean. Mann-Whitney *U*-test was used for statistical analysis. **P*< 0.05. CPT, calprotectin.

ileum before weight gain and increase in fat mass.[15]

Our results in adult groups are consistent with the reports by Mendall et al.[12] In a middle-aged healthy general population sample of 300 subjects (range, 50–70 years), they reported that the relationship of FC with BMI was not statistically significant, but the mean of FC in obese and overweight adult (BMI, 25.7–41.2 kg/m$^2$) was higher than that in non-obese and overweight (BMI, 17.6–25.6 kg/m$^2$).[16] What does it suggest that there is statistically not significant but difference between obese and overweight and non-obese and overweight groups? Recent report may be a clue to this matter. Verdam et al.[17] studied FC and gut microbiota composition of 28 adult (BMI, 18.6–60.3 kg/m$^2$) and found interesting finding that FC was only detectable in obese subjects with an obese pattern of microbiota. In contrary to adult groups, case control studies in pediatric groups are rare and a few reports about FC levels studied only in obese children. Spagnuolo et al.[18] studied 35 children with severe obesity (BMI >95%) and found that FC was increased only in 47% patients while CRP in 73.5%. Taken together, larger scale study and stratification based on other indicators such as gut microbiota composition than BMI is warranted.

The other interest finding in our result is that FC showed statistically significant difference between obese and overweight adult and obese and overweight children while FC showed no statistically significant difference between non-obese and overweight adults

and non-obese and overweight children.

Calprotectin is secreted extracellularly from stimulated neutrophils and the FC levels of healthy children exhibit a downward trend with increasing age and reach the adult level by the age of four.[19,20] This finding is consistent with recent FC study in healthy children aged less than 4 years in South Korea.[21] The children were divided into six age groups and the median FC values was 135 μg/g in 7–12 months group, 65 μg/g in 13–18 months group, 55 μg/g in 19–24 months group, 40 μg/g in 25–30 months group, 21 μg/g in 31–36 months group, and 12 μg/g in 37–48 months group. Why FC varies with age remains uncertain, but several research papers may be clues to this question and significant FC difference between AO and PO in our result. Lee et al.[22] studied FC levels of 133 healthy infants aged 0–6 months according to feeding mode and found that the mean FC values for breast-fed infants (354.67 μg/g) was higher than formula-fed infants (149.44 μg/g, $P < 0.001$).

Formula-fed boys (38.6%) were significantly more likely to be obese than breast-fed boys (23.4%, $P < 0.01$).[23] The ontogeny study of the immune system by type of infant feeding showed that blood cells of lymphoid lineage did not change significantly in frequencies or composition from 1.5 to 6 months of age in breast-fed infants. In contrast, formula-fed infants displayed an ongoing maturation of adaptive immunity cells and a delayed recruitment of innate immunity cells as compared with breast-fed infants.[24] Obesity is a well-established risk factor for venous thromboembolism in adults in contrary to children.[25] Taken these together, our finding and the above reports suggest different pathophysiologic background between adult obesity and children obesity. One possible explanation involves adaptive immunity behind children obesity and innate immunity behind adult obesity, and it warrants further research to elucidate exact mechanism and associated links between host gut immunity and obesity as inflammation. In conclusion, FC level in AO was significantly higher than that in PO, suggestive of different pathophysiologic mechanism between childhood and adulthood obesity. Further study is needed.

## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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