Roles of physical activity and cardiorespiratory fitness on sex difference in insulin resistance in late elementary years

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INTRODUCTION

Previous studies reported sex difference in body composition in late elementary school children; boys had greater fat-free mass and less fat mass than girls [1]. Body composition difference between boys and girls is primarily attributed to sex differences in secondary sex characteristics from puberty. In general, the average age of sexual maturation, which is the second stage of genital development in puberty, is 12-13 for boys and 10-11 for girls [2,3]. In general, girls experience an earlier manifestation of secondary sex characteristics compared to boys, marking the acceleration of sexual maturation around late elementary school years and contributing to a rapid increase in fat mass in girls [4].

Sex difference in body composition may become a serious health concern due to its relation to the elevated risk for chronic disease in childhood. In a recent study involving elementary school children, Kang et al. [5] reported that girls had unfavorable metabolic profiles in conjunction with greater sexual maturation and body fat than boys, implying an urgent need for lifestyle intervention to prevent metabolic diseases, especially in girls.

[Key words] children, body composition, insulin resistance, physical activity, physical fitness

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Untreated, excessive body fat would result in abnormal body fat accumulation, leading to an ectopic fat accumulation in insulin sensitive tissues such as the liver, skeletal muscle and thereby contributing to elevated risks for insulin resistance syndrome (IRS) and type 2 diabetes (T2D).

Several explanations have been given to explain the underlying mechanisms by which excessive body fat accumulation causes IRS and T2D. In recent, special attention has been paid to the potential role of cytokines secreted from body fat. Body fat functions as a storage for excessive energy and endocrine system [6]. Although body fat is involved in maintaining insulin sensitivity by regulating fat and carbohydrate metabolism [7], excessive body fat causes a low level of chronic inflammation due to an imbalance between insulin sensitive cytokines vs. insulin resistance cytokines. C-reactive protein (CRP), tumor necrosis factor-α (TNF-α), IL-6, retinol binding protein-4 (RBP-4), and leptin, which are secreted in response to excessive fat accumulation, are call insulin resistance cytokines [8-11], collectively decreasing whole body insulin sensitivity secondary to an inflammatory response. The inflammatory response causes the function loss of the buffering action of fat tissues and thereby insulin resistance in insulin sensitive tissues such as the liver, skeletal muscle, and pancreas, leading to the development of T2D [12].

Numerous studies have been conducted on the specific roles of the hormones that affect the amount of body fat secreted based on insulin resistance. Leptin, which is an obese gene protein produced from fat tissues, is a representative feeding inhibitor that causes feeding inhibition and thermogenesis enhancement normally by affecting the hypothalamus and centering about the arcuate nucleus. Leptin increases energy consumption by stimulating fatty acid oxidation [13]. Nevertheless, serum leptin level is higher in overweight or obese patients than normal weight people [14], and it is known as a compensatory function in response to decreased leptin sensitivity [15,16].

Adiponectin is an insulin sensitive cytokine secreted from adipose tissue. Serum levels of reported to be lower in overweight or obese patients than in normal people. Unlike leptin, however, adiponectin enhances fatty acid oxidation by activating AMP activated protein kinase (AMPK), which plays a key role in intracellular carbohydrate and fat metabolism in skeletal muscle and the liver by increasing the tyrosine phosphorylation of insulin receptors. Adiponectin reduces the deposition of triglycerides in muscles and the liver, and thereby it minimizes lipotoxicity [17]. Along with the activation of AMPK, adiponectin increases fat burning and energy dissipation by increasing the expression of genes related to fatty acid transport and oxidation in skeletal muscles (CD36, acyl-CoA oxidase, and uncoupling protein). In addition, adiponectin minimizes the deposition of triglycerides and ultimately plays a role in overcoming insulin resistance [18]. Yamauchi et al. [19] found that administration of the physiological concentration of adiponectin resulted in improved insulin resistance in conjunction with decreased free fatty acid and triglycerides in circulating serum in leptin and adiponectin genes-knockout mice. It was particularly interest to find that although administration of leptin alone partially improved insulin resistance, simultaneous administration of leptin and adiponectin recovered insulin resistance fully [19]. Consequently, the findings of the study suggest that both adiponectin and leptin are essential cytokines to prevent and/or treat IRS and T2D.

PA-induced increase of energy expenditure is a safe and effective means to reduce body fat and maintain optimal body weight for children and teenagers undergoing puberty and sexual maturation [20] as well as a non-pharmacologic strategy to prevent and/or treat IRS and T2D [21]. In addition to PA, cardiorespiratory fitness is associated with decreased leptin and elevated adiponectin in circulation. With respect to energy metabolism, cardiorespiratory fitness is defined as aerobic metabolism that delivers oxygen to skeletal muscle with a synergistic action of the lungs, heart and vascular system. Therefore, poor cardiorespiratory fitness secondary to sedentary lifestyle and physical inactivity is associated with decreased energy consumption and excessive accumulation of triglycerides in fat and muscle tissues. On the other hand, high cardiorespiratory fitness is associated with low fat mass and favorable blood lipids profile [22]. In addition, cardio respiratory fitness contributes to increased insulin sensitivity in peripheral tissues such as skeletal muscle by maintaining glucose homeostasis [23] and stimulating lipid oxidation and mitochondrial function [24].

Despite previous findings, few studies are available about the roles of PA and cardiorespiratory fitness on sex differences in insulin resistance in late elementary school children in Korea. Therefore, this study investigated the role of PA and cardiorespiratory fitness on sex difference in insulin resistance and metabolic risk factors in late elementary school children.

**METHODS**

**Study participants**

Study participants were consisted of boys and girls aged 12-13 years who attended an elementary school located in...
Gyeonggi-do. Apparently healthy elementary school students not taking drugs that affect blood pressure, blood sugar, lipids and weight were chosen based on basic assessment data. The content, goal and related procedures of the study were fully explained to the test participants and their parents. Informed and signed consent was obtained before participation in the study. Data on 150 students (81 girls and 69 boys), including physical assessment, biochemical test, cardiorespiratory fitness and the amount of PA for 7 days, were successfully measured and statistical analysis was performed.

Measurement and analysis items

Measurement of all dependent variables was done on an empty stomach (after 12 hours of fasting). Height and weight were measured in accordance to a standardized guideline. The participants wore comfortable clothes during the assessment.

Physical characteristics

While wearing thin clothes, weight was measured using automatic assessment equipment (DS-102, JEnix Co., Korea). Body fat percentage (%BF) was measured using the automatic body composition analyzer, X-Scan Body Composition Analyzer (Jawon Medical Co., Korea), utilizing bioelectrical impedance. BMI (body mass index) was obtained using the formula \[ \text{BMI} = \frac{\text{weight (kg)}}{\text{height (m)}^2} \]. WC was obtained by measuring twice with a tape measure horizontally across the navel region at the end of expiration in a standing position. The mean was used for analysis.

Sexual maturation (Tanner scales) was categorized into 5 stages for boys (based on genital, including penis and scrotum, and pubic hair development) and girls (based on breast and pubic hair development). The categories were pre-puberty (1st stage), the beginning of puberty (2nd stage), puberty (3rd stage), the end of puberty (4th stage) and adult period (5th stage) [25]. Sexual maturation was investigated using a questionnaire with 5 stages of penis and pubic hair pictures for boys and 5 stages of breast and pubic hair pictures for girls. Maturation development stage was measured with a 1 to 5 point system, with higher scores for more advanced sexual maturation.

Insulin resistance factor

After 12 hours of fasting, 8ml of venous blood from the brachial vein of the participants was obtained to measure the level of blood lipids and blood sugar. Serum was separated by centrifugation with Green Vac-Tube (3000rpm, 15 minutes), which contained a gel-type coagulation activator. The serum was then stored frozen in a -80 °C ultra low temperature freezer. The stored serum was used for the analysis of triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and fasting blood glucose (FBG) using Vitro Chemistry DT60 II (Johnson & Johnson, NY, USA). Insulin was measured using a Human Insulin ELISA kit (ALPCO Diagnostics) (the CVs for intra- and interassays = 4.3% and 6.8%, respectively). HOMA-IR (Homeostasis model assessment of insulin resistance) was calculated by \[ \text{HOMA-IR} = \frac{\text{fasting insulin (μU/mL) } \times \text{ fasting glucose (mM)}}{22.5} \] as proposed by Matthews, etc. [26].

Serum leptin and adiponectin

Serum leptin was analyzed using a Human Leptin ELISA kit (DSL, Texas, USA) (the CVs for intra- and interassays = 3.2% and 4.4%, respectively) and adiponectin was analyzed using a Human Adiponectin ELISA kit (AdipoGen, Seoul, Korea).

Cardiorespiratory fitness

Maximal oxygen consumption (VO₂max) was used to quantify cardiorespiratory fitness and was measured with a graded treadmill walking and/or running Bruce protocol. Specifically, the speed of the treadmill was initially set at the subject’s comfortable speed (walking 1-1.7 mph) at a 10% grade, with speed and grade increased every 3 min until volitional exhaustion (average final speed of 4 ± 0.4 mph and average final grade of 15.5 ± 0.9%). Requirements to determine whether subjects reached their VO₂max by this protocol included at least 2 of the following 4 criteria (2): (i) leveling off of VO₂, (ii) rate of perceived exertion (RPE) greater than 17 using the original category scale, (iii) volitional exhaustion, and (iv) reaching age-predicted maximal heart rate. A plateau in VO₂ was defined as a change of < 2 mL/min/kg in VO₂ over the last 60 s of the test. The subjects were verbally encouraged to exercise to exhaustion during the test. All subjects reached VO₂max, 97.8% had a respiratory exchange ratio (RER) > 1.0 and fulfilled the second criterion, and 80% of participants achieved 3 or all 4 of the criteria.

Physical activity (PA)

PA was assessed with the Kenz Lifecorder EX, a uniaxial accelerometer (LC; Suzuken Co. Ltd, Nagoya, Japan). All children were asked to wear the device from the time they got up in the morning until they went to bed at night, except during bathing and showering, for the full 7-day data collection period. At the end of the seventh day, the research staff manually stopped the LC, and the recorded data were downloaded to a personal computer. The activity levels were
categorized into one of nine activity classes (levels 1.0-9.0) based on PA energy expenditure. The nine activity levels were further classified into light PA (LPA), moderate PA (MPA), and vigorous PA (VPA) [27].

Statistics

Data are presented as means ± standard deviation (SD). Data were checked for normal distribution prior to data analysis. If skewed, transformed log10 was applied. Independent t-tests were used to test any significant group differences in the measured variables between boys and girls. Data analyses were performed with SPSS-PC (version 18.0) at a statistical significance of 0.05.

RESULTS

Sex differences in physical characteristics and body composition

[Table 1] represents the physical characteristics of the participants in the study. Boys had significantly higher BMI ($p = 0.019$) and WC ($p < 0.001$) than girls, with no significant difference in mean age. In addition, girls had significantly higher body fat ($p < 0.001$) and sexual maturation ($p < 0.001$) than boys.

Sex differences in blood lipids and insulin resistance markers

[Table 2] represents insulin resistance markers of boys and girls. Girls had significantly higher triglyceride ($p = 0.005$), insulin ($p < 0.001$) and HOMA-IR ($p < 0.001$) than boys. On the other hand, boys had significantly higher high-density lipoprotein cholesterol ($p = 0.015$) than girls, with no statistically significant differences in total cholesterol ($p = 0.092$) and fasting blood glucose ($p = 0.879$).

Sex differences in serum leptin and adiponectin

[Table 3] represents serum leptin and adiponectin of boys and girls. Girls had significantly higher serum leptin levels ($p = 0.014$) and lower serum adiponectin level ($p = 0.001$) than boys.

Table 1. Physical characteristics of girls (n = 81) and boys (n = 69)

| Group | Mean ± SD | 95% CI | $P$ value |
|-------|-----------|--------|-----------|
| Age (years) | Girls | 12.6 ± 0.5 | 12.5-12.6 | 0.087 |
| | Boys | 12.6 ± 0.4 | | |
| BMI (kg·m⁻²) | Girls | 19.3 ± 3.0 | 19.4-20.5 | 0.019 |
| | Boys | 20.7 ± 3.3 | | |
| Body fat (%) | Girls | 21.6 ± 5.5 | 18.1-20.3 | < 0.001 |
| | Boys | 16.3 ± 6.8 | | |
| WC (cm) | Girls | 70.4 ± 8.0 | 70.1-73.0 | < 0.001 |
| | Boys | 73.0 ± 9.9 | | |
| Tanner scale | Girls | 2.0 ± 1.0 | 1.6-1.9 | < 0.001 |
| | Boys | 1.4 ± 0.7 | | |

Table 2. Blood lipids and insulin resistance markers of girls (n = 81) and boys (n = 69)

| Group | Mean ± SD | 95% CI | $P$ value |
|-------|-----------|--------|-----------|
| TC (mg·dL⁻¹) | Girls | 185.8 ± 40.0 | 174.2-187.2 | 0.092 |
| | Boys | 174.7 ± 40.0 | | |
| TG (mg·dL⁻¹) | Girls | 110.8 ± 42.7 | 92.5-108.3 | 0.015 |
| | Boys | 88.1 ± 53.2 | | |
| HDLC (mg·dL⁻¹) | Girls | 47.7 ± 10.6 | 48.0-52.0 | < 0.001 |
| | Boys | 52.7 ± 13.6 | | |
| FBG (mg·dL⁻¹) | Girls | 91.2 ± 14.7 | 88.7-93.4 | < 0.001 |
| | Boys | 90.9 ± 14.7 | | |
| Insulin (uU·mL⁻¹) | Girls | 12.2 ± 7.4 | 9.1-11.3 | < 0.001 |
| | Boys | 7.9 ± 5.5 | | |
| HOMA-IR | Girls | 2.76 ± 1.74 | 2.04-2.57 | < 0.001 |
| | Boys | 1.77 ± 1.13 | | |

Table 3. Serum leptin and adiponectin levels of girls (n = 81) and boys (n = 69)

| Group | Mean ± SD | 95% CI | $P$ value |
|-------|-----------|--------|-----------|
| Leptin (ng·mL⁻¹) | Girls | 8001 ± 4569 | 6136-7891 | 0.014 |
| | Boys | 5853 ± 4917 | | |
| Adiponectin (mg·L⁻¹) | Girls | 10.5 ± 7.9 | 7.7-9.9 | 0.001 |
| | Boys | 7.0 ± 4.5 | | |

Table 4. VO₂max and accelerometer-based physical activity of girls (n = 81) and boys (n = 69)

| Group | Mean ± SD | 95% CI | $P$ value |
|-------|-----------|--------|-----------|
| VO₂max (mL·kg⁻¹·min⁻¹) | Girls | 39.1 ± 5.8 | 40.3-42.6 | < 0.001 |
| | Boys | 44.2 ± 7.9 | | |
| LPA (min·day⁻¹) | Girls | 87.5 ± 24.3 | 106.0-139.2 | < 0.001 |
| | Boys | 163.8 ± 139.3 | | |
| MPA (min·day⁻¹) | Girls | 33.5 ± 10.8 | 42.6-57.2 | < 0.001 |
| | Boys | 69.1 ± 60.4 | | |
| VPA (min·day⁻¹) | Girls | 10.6 ± 6.3 | 16.5-25.3 | < 0.001 |
| | Boys | 32.9 ± 36.3 | | |

LPA: low physical activity; MPA: moderate physical activity; VPA: vigorous physical activity
Sex differences in cardiorespiratory fitness and physical activity

[Table 4] represents cardiorespiratory fitness and physical activity of boys and girls. Boys had significantly higher VO_{2max} (p < 0.001) as well as accelerometer-based low- (p < 0.001), moderate- (p < 0.001), and vigorous-intensity PA (p < 0.001) than girls.

DISCUSSION

This study investigated sex differences in physical characteristics, blood lipids, insulin resistance factor, serum leptin and adiponectin in boys and girls (N = 150) aged 12-13 in late elementary school grades. In this study, we found that boys had larger physiques (e.g., BMI) and WC (p < 0.001) than girls. On the other hand, girls had greater body fat (p < 0.001) and sexual maturation (p < 0.001) in conjunction with higher values in triglyceride (p = 0.005), insulin (p < 0.001), and HOMA-IR (p < 0.001) and lower high-density lipoprotein cholesterol (p = 0.015) than boys. Of particular, it was interest to find that girls had a significantly higher risk for insulin resistance than boys.

Several explanations can be given for the sex difference in insulin resistance.

First, girls had greater fat accumulation secondary to greater sexual maturation than boys, leading to greater amount of ectopic fat accumulation associated with insulin resistance.

Second, girls had lower amounts of PA at all intensity levels, including low intensity (p < 0.001), moderate intensity (p < 0.001) and vigorous intensity (p < 0.001) than boys. In addition, girls had had relatively lower cardiorespiratory fitness (p < 0.001) than boys. Consequently, physical inactivity and poor cardiorespiratory fitness would contribute to a greater risk for insulin resistance in girls vs. boys.

Third, girls had lower adiponectin levels (p < 0.001) and higher leptin levels than (p = 0.014). Adiponectin is an insulin sensitive cytokine, and leptin is an insulin resistance cytokine. Collectively, decreased adiponectin and increased leptin would contribute to elevated risk for insulin resistance in girls.

Based on the current findings of the study, girls experienced excessive accumulation of body fat due to fast developing secondary sexual characteristics. Thus, girls experienced higher increase in blood insulin levels due to insulin resistance. In addition to the physical changes, girls had lower PA than male students, which may have created a vicious cycle where lack of PA leads to decreased physical fitness, leading to increased blood insulin and so on. Although our study did not quantify nutritional intake, it could be one of the main variables, which remain to be confirmed in future studies.

Puberty in late elementary school children begins with the synthesis and secretion of sex hormones. This is accompanied by the multiplication of reproductive cells in human gonads due to the increased secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH) in the pituitary gland caused by gonadotropin releasing hormone (GnRH) in the hypothalamus.

The manifestation of glandular tissue development is the first sign of puberty in female children (breast stage 2, B2) [28]. The median B2 age has become younger in many countries as shown in the decrease from 10.9 years old in 1985 [29] to 9.7 years old by 1994 in the United States [30] and from 10.88 years old in 1991 to 9.86 years old by 2008 in Denmark [31]. The mean age of menarche also dropped as shown in the decrease from 13.5 years old in 1979 to 12.27 years old by 2005 in Chinese female children [32]. Park, et al. [33] reported that the mean age of menarche for Korean female children was 2 years younger than the mean age 80 years ago. Also, puberty is a time of testicular volume increases of 4 mL or higher in male children (genital stage 2, G2). The onset of puberty in male children also occurs at a younger age as shown in the G2 median age of 11.5 years old in 1985 [29] dropping to 10.1 years old by 1994 in American Caucasian male children [34]. The onset of puberty also dropped from 12.7 years old [35] in 1992 to 10-11 years old by 2008 in Korean male children [36]. Nutritional intake improvement and fat mass increase have been proposed as the reasons behind this trend of early onset of puberty [37-39].

The age of puberty onset has decreased globally since the 1980s [40]. Therefore, in a study of critical fat mass around the onset of menarche in domestic late elementary school aged female students, fat mass was 17-19% and had a higher correlation to fat percentage and fat mass than WC and hip circumference [41]. It was confirmed that the mean critical fat mass of female students served as an intermediate variable for the phenomenon of faster sexual maturation. Especially, body fat distribution was significantly higher in female children and the amount of exercise was implicated as one of the important factors affecting the change of body fat and lipids.

Kong [42] reported that overall PA level was lower in female children upon analyzing the fat mass and PA of 234 Korean children (116 male students and 118 female students) aged 10 to 12. The National Youth Policy Institute [43] reported that there were sex-dependent differences regarding the participation ratio in vigorous PA 3 times a week, with 41.6% of male students and 16.7% of female students.
participating. Especially, female students lacking in PA had relatively higher fat mass than male students and also had twice the insulin resistance (100%) [5,44,45]. In other words, fat mass is an obvious risk factor affecting the onset of insulin resistance in children. The results can be interpreted as indicating that female students need preferential intervention to actively control body fat. Upon comparison of PA, blood lipids and insulin resistance markers based on gender in 44 obese male children and 34 female children in 5-6 grades in elementary school, Hah et al. [46] reported that low, moderate and vigorous PA were significantly higher in male children. The study also reported that total cholesterol and triglyceride were significantly higher in female children but that high-density lipoprotein was significantly lower in female children.

Hah et al. [46] reported that upon conducting additional comparative analysis on blood lipids and insulin resistance markers based on gender in 39 children (24 male children and 15 female children), who practiced moderate to vigorous PA for at least 60 minutes every day, which is the amount recommended by ACSM [47], there was no statistically significant difference in total cholesterol, triglyceride, high-density lipoprotein cholesterol, fasting blood sugar, insulin and HOMA-IR between the male and female children. On the other hand, the group that did not practice moderate to vigorous PA showed significantly higher total cholesterol and insulin resistance markers (Insulin, HOMA-IR) in female children. However, the female children had significantly lower high-density lipoprotein cholesterol. In other words, insulin resistance risk factors appear to be affected by the intensity of PA and the practice of moderate to vigorous PA seems to positively influence the prevention of metabolic disease regardless of gender.

Furthermore, according to previous studies that reported regular exercise improving the imbalance of insulin resistant and insulin sensitive cytokines caused by obesity, the serum leptin level response was generally affected by exercise intensity and duration [48,49]. However, the level of serum leptin decrease, based on the practice of the exercise program, was significantly higher in obese patients than in normal weight people [50,51]. Pasmem et al. [52] reported that after the implementation of endurance training (moderate intensity) for 1 hour, 3 to 4 times a week for 16 months on 15 obese men (mean age was 37.3 years old and BMI was 28.9), serum leptin level was independently reduced regardless of the change in insulin or fat mass. Kondo et al. [53] also reported increased adiponectin along with insulin resistance improvement after exercise training for 7 months in obese women. Most studies have reported that short-term and long-term exercise training improved insulin and also raised serum adiponectin levels [54].

To summarize the previous studies, there was no consistency in the response of serum adiponectin regarding the long-term maintenance or the decrease. However, based on the results of previous studies reporting generally significant increases in serum adiponectin levels by regular exercise on a consistent basis, regular practicing of moderate to vigorous PA plays a positive role in cardiorespiratory fitness improvements such as fat mass and blood lipids improvement [22] as well as increased heart function to transport oxygen to muscles [55].

Dencker et al. [56] emphasized the importance of continuous moderate to vigorous PA in children since VO2peak was higher if the amount of moderate to vigorous PA, lasting longer than 10 minutes, was higher. They also mentioned the importance of continuous moderate to vigorous PA as well as overall PA amount. Twisk et al. [22] reported in their study that the amount of PA is not only related to VO2max but also closely related to total cholesterol, high-density lipoprotein cholesterol and body fat. We attribute such results to the positive effect on elasticity and resistance of blood vessels [57] caused by the improvement of blood sugar utilization in skeletal muscles due to PA. Blood circulation and heart function are also improved, as PA encompasses all activities related to contraction of the musculoskeletal system. In conclusion, we found that girls had a greater risk for metabolic conditions such as obesity, IRS and T2D than boys. This greater risk for metabolic disease in girls is associated with greater sexual maturation and body fitness, physical inactivity and poor cardiorespiratory fitness, and lower adiponectin and higher leptin in circulating serum compared to boys. Consequently, the findings of the current study suggest that promotion of daily physical activity and cardiorespiratory fitness are imperative as a safe and effective means to prevent and/or treat metabolic risk factors, especially in girls.

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