

Differences in Adipokine and Hepatokine Levels among Non-diabetic Population Classified by Age and Sex

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Background: Leptin, angiotensin-related growth factor (AGF), adiponectin (ADP), and retinol-binding protein 4 (RBP4) are cytokines associated with the development of metabolic disorders, such as type 2 diabetes and cardiovascular disease. However, the levels of these cytokines have not extensively studied in non-diabetic subjects. Therefore, we analyzed the differences in these cytokine levels according to sex and age in non-diabetic Korean population.

Methods: Blood samples were collected from 59 non-diabetic Korean adults (male, 32; female, 27). The anthropometric and biochemical data were measured at the health examination center. Serum adipokines and hepatokines were measured by enzyme linked immunosorbent assay (ELISA). The data were analyzed according to sex and age-based quartiles.

Results: Serum leptin values were higher in females ($8.60 \pm 3 \mu\text{g/ml}$) compared with males ($2.99 \pm 2.9 \mu\text{g/ml}$). However, RBP4 was higher in males ($84.05 \pm 47.04 \mu\text{g/ml}$) than in females ($61.25 \pm 45.42 \mu\text{g/ml}$). The AGF and ADP values were not significantly different between males and females. RBP4 level was inversely correlated with age quartile in males, while leptin was significantly associated with body mass index and insulin resistance.

Conclusion: RBP4 and AGF levels showed age-associated change, and leptin was consistently higher in females. Therefore, a large-scale analysis to determine the normal range of adipokines and hepatokines concentration in healthy Korean population is necessary. When interpreting adipokine and hepatokine levels, the difference in age and sex needs to be taken into account.

Key Words: Adiponectin (ADP), Angiotensin-related growth factor (AGF), Retinol-binding protein 4 (RBP4), Leptin, Non-diabetic subjects

INTRODUCTION

The incidence of obesity is dramatically increasing across the world, and it is a contributor to glucose intolerance, hypertension, and cardiovascular disease [1]. Recently, adipokine and hepatokine levels have been viewed as important

markers in predicting metabolic syndrome in subjects with obesity and type 2 diabetes. Adipokines and hepatokines are secreted from adipose tissue and liver [2,3], respectively. Over the last few decades, adipose tissues have been well known not only as mere storehouses of fuel but also as secretors of a wide variety of hormones. Adipose tissue, which is increased in obesity, stores fat and secretes several cytokines, such as tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , IL-6, leptin, adiponectin (ADP), and resistin [4,5].

Leptin is possibly the first adipokine associated with direct pancreatic effects and is certainly the most studied of all adipokines with respect to its pancreatic effects. It is now

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generally accepted that leptin has a potent inhibitory effect on insulin secretion from pancreatic β -cells *in vitro* and *in vivo* has an additional effect on reducing pre-proinsulin gene expression [6]. Moreover, it also regulates energy intake [7] and energy expenditure. Meanwhile, ADP has a beneficial effect on improving insulin sensitivity and vascular function. The secretion of ADP is reduced with increasing adiposity. ADP modulates glucose regulation and fatty acid oxidation in the cells [8]. Another adipokine, retinol binding protein (RBP4), which was known previously as transporter of retinol (vitamin A), is also secreted from the adipose tissue. It induces insulin resistance in the liver and skeletal muscle of mice [5]. Human studies have showed a correlation between RBP4 and insulin resistance [5-9], and RBP4 is also strongly associated with metabolic syndrome components [10].

Angiopoietin-related growth factor (AGF), also known as Angptl 6, has been introduced as a novel liver-derived protein called hepatokine that antagonizes obesity and insulin resistance. According to one study, AGF-knockout mice showed noticeable obesity and insulin resistance, including lipid accumulation in skeletal muscle and liver [9]. AGF increases energy expenditure and improves insulin sensitivity and lipid profiles [11]. There is a well-established relationship between adipokine and hepatokine in type 2 diabetes population. However, there is a paucity of studies on adipokines and hepatokines in respect to different age groups in non-diabetic populations.

Therefore, we measured serum adipokine and hepatokine levels in non-diabetic subjects in male and female according to age quartiles.

MATERIALS AND METHODS

1. Subjects

This study involved 59 non-diabetic Korean adults (male = 32, female = 27) who visited the health examination center in Wonju Severance Christian Hospital. All participants agreed to the use of their clinical data and gave their written informed consent for this study. The study was approved by the Institutional Review Board at Yonsei University Wonju College of Medicine. Exclusion criteria for study subjects were as follows: subjects with diabetes, those younger

than 30 years or older than 70 years, and those who had serum creatinine (Cr) greater than 1.5 mg/dl.

2. Anthropometric and biochemical markers

Anthropometric factors and biochemical markers were measured at the health examination center. Body mass index (BMI) was calculated by the BMI formula.

$$\text{BMI (kg/m}^2\text{)} = \text{Body Weight (kg)} / \text{Height}^2 \text{ (m}^2\text{)}$$

Serum levels of ADP, AGF, and RBP4 were determined with enzyme linked immunosorbent assay (ELISA) kit (Adipogen; Adipogen Inc., Seoul, Korea). Leptin level was also analyzed by ELISA kit (R&D Systems, Minneapolis, MN).

3. Sex and age quartile

The subjects were classified into a total of four groups according to sex and age quartiles: Q1, 30-39 years (average age of male: 35.75 years, average age of female: 35.43 years); Q2, 40-49 years (average age of male: 43.2 years, average age of female: 45.71 years); Q3, 50-59 years (average age of male: 54.25 years, average age of female: 55.14 years); Q4, 60-69 years (average age of male: 64.25 years, average age of female: 63.50 years).

4. Statistics

The results were expressed as mean \pm standard deviation (SD). The data were analyzed by using SPSS software version 18.0 (SPSS Inc., Chicago, IL, USA). Analysis of variance (ANOVA) was used to compare the mean of adipokines and hepatokines according to age quartiles. Student's t-test was used to compare the adipokines and hepatokines mean values between males and females. Pearson's correlation coefficient was used to evaluate the relationship between adipokines, hepatokines and biochemical characteristics of the subjects. p-value < 0.05 was considered statistically significant.

RESULTS

1. Baseline characteristics of male and female subjects

The anthropometric and biochemical data of all subjects are summarized in Table 1. Height, body weight (BW),

Table 1. Comparison of biochemical characteristics in male and female

	Male (n=32)	Female (n=27)	p
Age (years)	49.44 ± 11.31	49.44 ± 10.82	NS
Height (cm)	169.91 ± 7.07	157.52 ± 6.62	NS
BW (kg)	70.88 ± 8.29	58.85 ± 8.97	NS
BMI (kg/m ²)	24.53 ± 2.24	23.74 ± 3.58	NS
FBS (mM/l)	99.74 ± 17.73	96.78 ± 9.78	NS
Insulin (mU/l)	5.66 ± 3.49	5.38 ± 2.60	NS
HOMA-IR	25.43 ± 15.60	23.26 ± 11.85	NS
GGT (U/l)	43.06 ± 42.51	18.70 ± 10.06	0.001
C-peptide (mg/dl)	1.11 ± 0.49	0.98 ± 0.27	0.027
TC (mg/dl)	196.81 ± 35.03	205.44 ± 44.09	NS
TG (mg/dl)	143.84 ± 85.64	106.07 ± 60.68	NS
HDL (mg/dl)	61.88 ± 14.81	62.59 ± 14.45	NS
LDL (mg/dl)	119.78 ± 32.50	130.22 ± 37.69	NS
AST (U/l)	26.84 ± 11.07	21.52 ± 5.26	0.046
ALT (U/l)	27.88 ± 19.12	20.15 ± 16.11	NS
Alcohol intake (g)	18.49 ± 26.39	4.64 ± 15.21	0.00

BW: body weight, FBS: fasting blood sugar, GGT: gamma-glutamyl transpeptidase, TC: total cholesterol, TG: triglyceride, AST: aspartate aminotransferase, ALT: alanine aminotransferase. Data expressed as mean ± standard deviation. Signifies p-value < 0.05 compared to the male.

gamma-glutamyl transpeptidase (GGT), and aspartate aminotransferase (AST) were significantly higher in male compared with female. Alcohol intake was also higher in male. Other parameters including BMI, fasting blood glucose, serum insulin, Homeostasis Model of Assessment-Insulin Resistance (HOMA-IR), triglyceride (TG), and alanine aminotransferase (ALT) appeared to be higher in males, but the differences were not found to be significant.

2. Differences of serum adipokine and hepatokine levels between male and female

The mean of serum adipokine and hepatokine concentrations are shown in Table 2. Although the sexual difference is mostly insignificant, the leptin and leptin-to-ADP ratio (L/A) were significantly higher in females than in males. In males, RBP4 was higher than in females while ADP and AGF were lower in males when compared with females, however, the difference was not statistically significant.

3. Comparisons of BMI between male and female according to the age quartiles

In age quartiles, BMI was higher in male Q1-3 than in

Table 2. Comparison of adipokine and hepatokine levels between male and female

	Male (n=32)	Female (n=27)	p
RBP4 (μg/ml)	84.05 ± 47.04	61.25 ± 45.42	0.06
ADP (μg/ml)	7.07 ± 3.84	8.23 ± 3.6	0.24
Leptin (ng/ml)	2.99 ± 2.9	8.6 ± 7.37	0.02*
L/A	0.59 ± 0.72	1.30 ± 1.18	0.04*
AGF (ng/ml)	77.42 ± 26.55	80.54 ± 24.05	0.64

RBP4: retinol binding protein 4, ADP: adiponectin, L/A: leptin to adiponectin ratio, AGF: angiotensin-related growth factor. The Data expressed as mean ± standard deviation. *signifies p-value < 0.05 compared to the male.

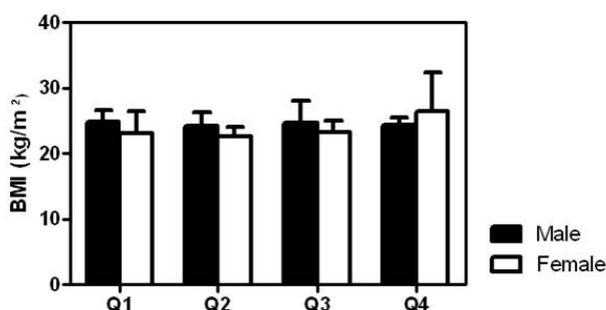


Fig. 1. BMI differences according to gender in age quartiles groups. The age were classified to four groups and compared with male and female in each age group. Q1: 30-39 years, Q2: 40-49 years, Q3: 50-59 years, Q4: 60-69 years. The Data expressed as mean ± standard deviation.

female Q1-3, but was similar in Q4 (Fig. 1).

4. Mean serum concentration of adipokine and hepatokine in male and female according to age quartiles

The comparison of adipokine and hepatokine levels according to each age quartile in male and female is shown in Fig. 2. RBP4 level was higher in males compared to females in all age quartiles, but the difference was significant only in Q1 (Fig. 2A). Adiponectin level was higher in Q1 of females but did not show any significance. In males, the level of adiponectin was found to be higher in Q2 and Q4, compared to female subjects of the same age quartile. Adiponectin levels of Q3 in females were significantly higher than in males (Fig. 2B). AGF level was not different among the age groups in either gender (Fig. 2C). Leptin level was found to be lower in all age quartiles of males,

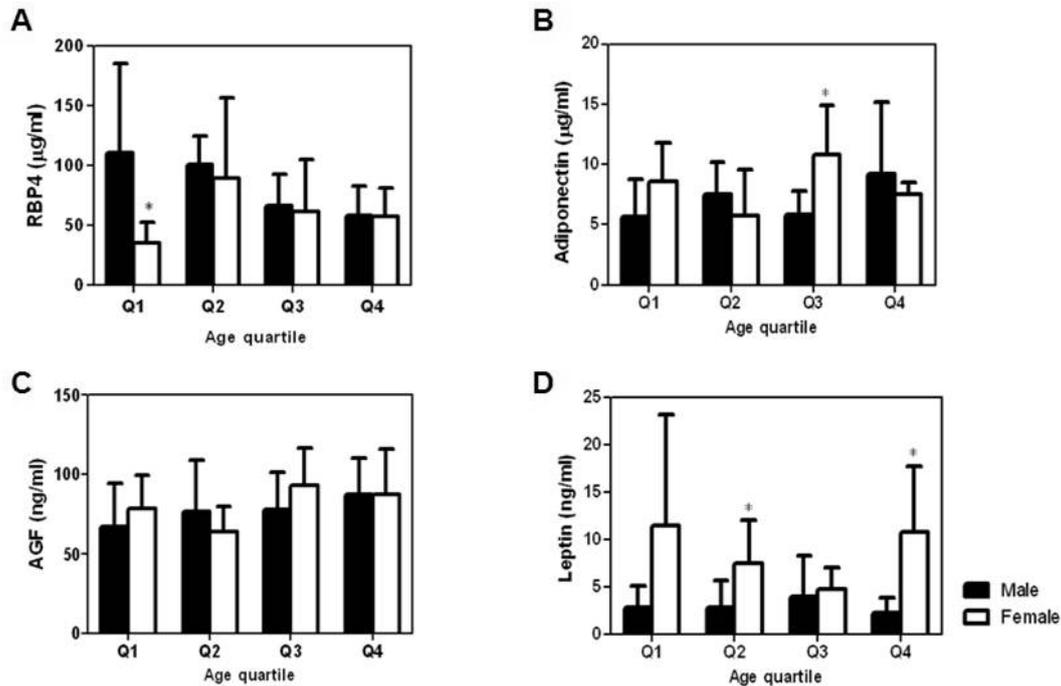


Fig. 2. Association of serum adipokine and hepatokine concentrations with age and gender. Blood serum was analyzed to compare the adipokine and hepatokine between males and females and determine their associations with age quartile. Q1: 30-39 years, Q2: 40-49 years, Q3: 50-59 years, Q4: 60-69 years. (A) Comparisons of serum RBP4 levels. (B) Comparisons of serum adiponectin levels. (C) Comparisons of serum AGF levels. (D) comparisons of serum leptin levels. The Data expressed as mean \pm standard deviation. *signifies p value < 0.05 compared to the male.

compared to females; In Q2 and Q4, leptin level was significantly higher in females than in male subjects (Fig. 2D).

5. Correlation analysis of anthropometric and biochemical markers associated with serum adipokine and hepatokine concentrations

The associations between anthropometric and biochemical markers with serum adipokine or hepatokine levels in whole subjects are depicted in Table 3, which was analyzed with Pearson's correlation coefficient test.

RBP4 showed positive correlation with smoking and alcohol intake. ADP was negatively correlated with TG but had positive correlation with hs-CRP. Leptin showed positive correlation with BMI and hs-CRP but had negative correlation with smoking, GGT, AST, and hemoglobin. L/A ratio showed positive correlation with BMI, insulin, HOMA-IR, and C-peptide. The level of AGF showed negative correlation with height.

6. Correlation between adipokine and hepatokine in all subjects

Table 4 represents the correlation between adiponectin and hepatokine levels. RBP4 was negatively correlated with ADP, leptin, and AGF. L/A was positively correlated with RBP4 and leptin but negatively correlated with ADP and AGF.

DISCUSSION

Previous studies demonstrated that obesity is correlated with BMI and that it is also closely related to adipocyte- or hepatocyte-secreted cytokines [12]. Fat cells in obesity cause chronic inflammation, contributing to metabolic dysfunction in obesity and insulin resistance [13]. In addition, hepatokines are known to antagonize obesity and insulin resistance. There is, however, no standard data based on Korean population showing the levels of adipokines and hepatokines in non-diabetic subjects. Age is one of the independent risk factors for insulin resistance [14]. Therefore,

Table 3. Correlation of biochemical markers with adipokine and hepatokines in all subjects

	RBP4		Adiponectin		Leptin		L/A		AGF	
	r	p	r	p	r	p	r	p	r	p
Age	-.184	.163	.205	.120	-.071	.593	-.099	.454	.242	.065
Sex	-.242	.065	.156	.238	.465 [†]	.000	.350 [†]	.007	.062	.640
Smoking	.333 [†]	.010	.126	.343	-.268*	.040	-.198	.132	-.232	.076
Exercise	.152	.250	.132	.320	-.071	.593	-.148	.264	-.066	.620
Alcohol	.367 [†]	.004	.084	.527	-.104	.433	.013	.923	-.107	.421
BP	-.015	.913	.245	.062	.020	.883	-.027	.837	.146	.269
Height	.177	.180	-.064	.632	-.194	.142	-.128	.335	-.295*	.023
Weight	.159	.228	-.101	.446	.205	.120	.174	.187	-.214	.103
BMI	.042	.753	-.069	.604	.457 [†]	.000	.355 [†]	.006	.007	.960
FBS	.037	.785	-.197	.138	.013	.923	.030	.825	.111	.405
Insulin	-.045	.739	-.158	.237	.336 [†]	.010	.295*	.025	-.093	.489
HOMA-IR	-.035	.795	-.198	.140	.331*	.012	.290*	.029	-.070	.605
GGT	.140	.292	-.156	.239	-.039	.768	.100	.451	-.025	.849
Cpeptide	.094	.482	-.180	.176	.274*	.038	.289*	.028	-.110	.410
TC	.059	.658	-.004	.978	.055	.679	.013	.921	.068	.610
TG	.158	.231	-.305*	.019	-.069	.603	.098	.460	.052	.697
HDL	.019	.889	.181	.171	-.062	.639	-.097	.463	-.120	.366
LDL	.070	.599	-.013	.920	.083	.534	.038	.775	.081	.540
AST	-.048	.717	.120	.366	-.132	.318	-.129	.331	.040	.762
ALT	-.019	.884	-.030	.822	.040	.765	.034	.797	-.004	.978
CRP	-.156	.238	.306*	.019	.323*	.013	.123	.353	-.010	.938

FBS: fasting blood sugar, GGT: gamma-glutamyl transpeptidase, TC: total cholesterol, TG: triglyceride, AST: aspartate aminotransferase, ALT: alanine aminotransferase, CRP: c-reactive protein, r: Pearson’s coefficient. Data expressed as mean ± standard deviation (N=59). *signifies p-value < 0.05. †signifies p-value < 0.01.

Table 4. Correlation analysis of the factors associated with adipokine and hepatokine

	RBP4		Adiponectin		Leptin		AGF		L/A	
	r	p	r	p	r	p	r	p	r	p
RBP4	1		-0.252	NS	-0.072	NS	-.337	0.009	0.135	NS
Leptin	-0.072	NS	-0.020	NS	1		-0.141	NS	.813	0.000
ADP	-0.252	NS	1		-0.020	NS	-0.005	NS	-.371	0.004
L/A	0.135	NS	-.371	0.004	.813	0.000	-0.173	NS	1	
AGF	-.337	0.009	-0.005	NS	-0.141	NS	1		-0.173	NS

RBP4: retinol binding protein 4, ADP: adiponectin, L/A: leptin to adiponectin ratio, AGF: angiotensin-related growth factor, r: Pearson’s coefficient. Data expressed as mean ± standard deviation (N=59). signifies p-value < 0.01.

we divided age into four quartiles for each sex.

In this study, we analyzed the biochemical characteristics, adipokines, hepatokines, and their correlations in four groups of non-diabetic subjects divided according to age quartiles and sex. BMI, blood glucose, and serum insulin were not significantly different between males and females. It is well known that serum GGT is elevated in hepatobiliary disorders. AST and ALT are also increased in cases of liver damage from a variety of causes [15]. Males consumed

more alcohol than females, and accordingly, serum GGT, ALT, and AST levels were higher in males. Hs-CRP is a well-known inflammatory marker and is a major risk factor for coronary artery disease [16]. It is also associated with obesity-related insulin resistance and diabetes. Moreover, hs-CRP have negative correlation with adiponectin [17]. Nonetheless, in our study, positive correlation between hs-CRP and adiponectin was observed.

Various studies have demonstrated that leptin levels are

correlated with body fat [18]. Leptin is a well-known adipokine which controls appetite and weight, but leptin resistance is characterized by overweight humans [19]. In this study, leptin was significantly lower in males compared to females, while BMI was lower in females than in males except for Q4. In all subjects, BMI showed positive correlation with leptin and L/A. Increased leptin level indicates its relationship with HOMA-IR, which is a marker of insulin resistance. RBP4, a hepatokine measured in this study, had inverse effect on leptin. The RBP4 level was higher in males probably due to alcohol intake. We found a decrease in RBP4 levels as the age quartile increased in males, and the same trend was observed in female leptin levels with the exception of Q4.

The reason behind the inverse relationship between RBP4 and leptin needs further investigation, as does the negative correlation between adiponectin and hs-CRP in non-diabetic subjects.

Therefore, a large-scale analysis to determine the normal range of adipokines and hepatokines concentrations in healthy and diabetic Korean population is needed. Furthermore, when interpreting adipokine and hepatokine levels, the differences due to age and gender need to be taken into account.

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