A higher score on the Aging Males’ Symptoms scale is associated with insulin resistance in middle-aged men

Nobuya Hamanoue1), Makito Tanabe1), Tomoko Tanaka2), Yuko Akehi1), Junji Murakami3), Takashi Nomiyama1), 2) and Toshihiko Yanase1), 2)

1) Department of Endocrinology and Diabetes Mellitus, Faculty of Medicine, Fukuoka University, Fukuoka 814-0180, Japan
2) Department of Bioregulatory Science of Life-related Diseases, Faculty of Medicine, Fukuoka University, Fukuoka 814-0180, Japan
3) Department of Preventive Medicine, Iizuka Hospital, Iizuka 802-0018, Japan

Abstract. An age-associated androgen decrease and its pathological conditions are defined as late-onset hypogonadism (LOH). Among the various symptoms associated with LOH, a visceral fat increase is strongly associated with relatively low levels of testosterone. However, few studies have investigated the relationship between the Aging Males’ Symptoms (AMS) scores and metabolic abnormalities. Thus, we aimed to clarify this relationship by investigating the relationship between AMS scores and various markers in blood. During routine health examinations in 241 middle-aged males (52.7±7.5 years of age, mean±SD), 150 males (62.2%) displayed higher AMS values than normal. No statistical association was observed between total AMS scores and any testosterone value. All mental, physical and sexual AMS subscales were significantly positively correlated with insulin levels and HOMA-IR. Only sexual subscale scores were significantly inversely associated with free or bioavailable testosterone level. Males with insulin resistance (HOMA-IR≥2.5) demonstrated significantly higher AMS scores than those with normal insulin sensitivity (HOMA-IR<2.5). AMS values were positively correlated with fasting blood glucose, insulin and HOMA-IR values. Interestingly, univariate and multivariate analyses revealed that HOMA-IR≥2.5 was a significant predictor for detection of moderately severe AMS values (AMS≥37), whereas AMS≥37 was not a predictor of metabolic syndrome by International Diabetes Federation (IDF) criterion. In conclusion, almost 60% of healthy male subjects displayed abnormal AMS scores. AMS values were not associated with testosterone values but rather were related to insulin resistance, particularly in subjects with moderately severe AMS values. Insulin resistance-related general unwellness might be reflected by AMS values.

Key words: Testosterone, Late-onset hypogonadism, Insulin resistance, Metabolic syndrome

DECREASED ANDROGEN LEVELS associated with aging cause various symptoms, termed late-onset hypogonadism (LOH) [1-3]. LOH is characterized by (i) somatovegetative symptoms and conditions, including weakness, fatigue, sleep disturbance, visceral obesity, sarcopenia and osteopenia (or osteoporosis), (ii) psychological symptoms, including disturbed sense of well-being, depressed mood, irritability and anxiety and (iii) sexual symptoms, including decreased libido and erectile dysfunction.

Visceral obesity has been proposed to be caused by vice circulation. Specifically, decreased androgen levels result in visceral obesity, which then leads to the suppression of gonadotropin and subsequent testosterone deficiency [4]. We have reported that male ARKO mice develop visceral obesity because of decreased energy expenditure [5]. Metabolic syndrome (MetS) is characterized by multiple cardiovascular risk complications, including visceral obesity, hypertension, glucose tolerance and dyslipidemia. MetS incidence is rapidly increasing and has become a significant public health problem [6]. We have demonstrated that among various testosterone values, including total testosterone (TT), free testosterone (FT) measured by analogue ligand radioimmunoassay (aFT), calculated FT (cFT), calculated bioavailable testosterone (cbT) and sex hormone-binding globulin (SHBG), a decrease in TT is the most valuable indicator of MetS in middle-aged males [7]. While the protective effect of testosterone replacement on cardiovascular events remains
controversial [8], the relationship between androgen deficiency and MetS has been well established [9].

To assess LOH, a self-report scale, Heinemann’s Aging Males’ Symptoms (AMS) scale [10], was developed in 1999 in Germany to quantify the health-related quality of life and symptoms of aging men [11]. The AMS scale contains 17 self-rating symptom-based questions with three subscales, including mind (5 questions), body (7 questions) and sexual (5 questions). Responses to each question are assigned a rating (1–5, none to extremely severe), and the total sum of all subscales provides a total score. Total scores can range from a minimum of 17 to a maximum of 85. Scores 17-26 are considered normal. Scores greater than 27, 37 and 50 are considered mild, moderately severe and severe, respectively. The AMS questionnaire assists in both the diagnosis of testosterone deficiency and monitoring of patients using testosterone replacement therapy. However, numerous reports have determined that AMS values are not specific to the detection of testosterone deficiency [12-16]. Thus, it is unclear what conditions are reflected by AMS values.

To our knowledge, the relationship between MetS and AMS values has not yet been investigated. Excessive visceral fat accumulation causes increased levels of inflammatory cytokines, such as IL6, TNFalpha and IL1beta, resulting in increased CRP levels [17]. Although one study reported that testosterone replacement in patients with LOH decreased CRP levels and improved AMS values [18], the relationship between insulin resistance and the AMS scale has not been demonstrated.

The present study was undertaken to investigate what conditions are reflected by AMS values. We analyzed the relationship between AMS scores and factors, including testosterone values and metabolic and inflammatory markers, in middle-aged male subjects during a routine health examination. We determined that AMS scores are not associated with testosterone values but rather are associated with insulin resistance and related conditions.

**Subjects and Methods**

**Subjects**

The Institutional Review Boards at Fukuoka University Hospital and Iizuka Hospital approved the study protocol. Written informed consent was obtained from all subjects for participation in the study. The first 304 subjects who visited the Department of Preventive Medicine at Iizuka Hospital for a health examination were recruited. Of these, 53 subjects who were taking medication for type 1 and/or type 2 diabetes mellitus or hyperlipidemia, as revealed by a questionnaire, were excluded. Subjects who were taking antihypertensive drugs and considered to be hypertensive were not excluded, even though their blood pressure (BP) levels on the examination day were normal. Among the remaining 251 individuals, two subjects who lacked waist circumference data and eight subjects who lacked several markers in blood were also excluded. Therefore, 241 asymptomatic and healthy men (aged 52.7 ± 7.4 years) having all of the data were finally analyzed.

**Evaluations of various markers**

The actual AMS questionnaire was shown in Table 1 [10]. The questionnaire contains 17 self-rating symptom-based questions with three subscales, including 5 mental, 7 physical and 5 sexual types of questions. All subjects in this study answered this questionnaire and responses to each question were assigned a rating (1–5, none to extremely severe). The total sum of all subscales provided a total score. Total scores can range from a minimum of 17 to a maximum of 85. Scores 17-26 are considered normal. Scores greater than 27, 37 and 50 are considered mild, moderately severe and severe, respectively. The AMS questionnaire assists in both the diagnosis of testosterone deficiency and monitoring of patients using testosterone replacement therapy. However, numerous reports have determined that AMS values are not specific to the detection of testosterone deficiency [12-16]. Thus, it is unclear what conditions are reflected by AMS values.

To our knowledge, the relationship between MetS and AMS values has not yet been investigated. Excessive visceral fat accumulation causes increased levels of inflammatory cytokines, such as IL6, TNFalpha and IL1beta, resulting in increased CRP levels [17]. Although one study reported that testosterone replacement in patients with LOH decreased CRP levels and improved AMS values [18], the relationship between insulin resistance and the AMS scale has not been demonstrated.

The present study was undertaken to investigate what conditions are reflected by AMS values. We analyzed the relationship between AMS scores and factors, including testosterone values and metabolic and inflammatory markers, in middle-aged male subjects during a routine health examination. We determined that AMS scores are not associated with testosterone values but rather are associated with insulin resistance and related conditions.

The first 304 subjects who visited the Department of Preventive Medicine at Iizuka Hospital for a health examination were recruited. Of these, 53 subjects who were taking medication for type 1 and/or type 2 diabetes mellitus or hyperlipidemia, as revealed by a questionnaire, were excluded. Subjects who were taking antihypertensive drugs and considered to be hypertensive were not excluded, even though their blood pressure (BP) levels on the examination day were normal. Among the remaining 251 individuals, two subjects who lacked waist circumference data and eight subjects who lacked several markers in blood were also excluded. Therefore, 241 asymptomatic and healthy men (aged 52.7 ± 7.4 years) having all of the data were finally analyzed.
Serum adiponectin levels were measured with a Human adiponectin ELISA kit (Otsuka Pharmaceutical Co., Ltd., Tokyo Japan).

From the global point of view, MetS in males was diagnosed according to the IDF, 2009 version [21]. The IDF criterion require three or more of the following conditions: i) elevated systolic and/or diastolic BP of ≥130/85 mmHg, ii) an elevated FBG level of ≥100 mg/dL, iii) an elevated serum TG level of ≥150 mg/dL, iv) a decreased HDL-C level of <40 mg/dL and v) abdominal obesity with a waist circumference of ≥90 cm.

### Statistical analysis

Data are expressed as means ± standard deviation. Comparative analyses of continuous variables between two groups were performed, as appropriate, using an unpaired t-test. For comparison among groups, ANOVA with ad hoc Fisher’s least significant difference method were performed. Binary logistic regression analyses were executed to determine predictors for AMS≥37 and MetS, respectively, and the odds ratio with a 95% confidence interval were calculated by both univariate and multivariate analyses. When there was colinearity between two variables, either variable was excluded from the multiple logistic regression models. All statistical analyses were performed using IBM SPSS version 18.0. Values of p<0.05 were considered statistically significant.
Results

The mean total AMS value was 31.7±10.0 (n=241). There were 91 (37.8%), 86 (35.7%), 48 (19.9%) and 16 (6.6%) men with normal (17–26), mild (27–36), moderately severe (37–49) and severe (≥50) AMS scores, respectively and 62.2% displayed abnormal scores (≥27) (Fig. 1).

The correlation coefficients between total AMS values and testosterone values, including TT, aFT, cFT, cbT and SHBG, were -0.097, -0.082, -0.096, -0.112 and -0.017, respectively, and no significance was observed for any combination (Table 2). Total AMS scores were positively and significantly correlated with FBG levels (r=0.159, p=0.0135), insulin levels (r=0.184, p=0.0042) and HOMA-IR (r=0.217, p=0.0007) but not age, BMI, waist circumference or LDL-C, HDL-C, TG, HbA1c, HOMA-β or adiponectin levels (Table 2). AMS scores tended to display a weak positive correlation with log hs-CRP levels, but it was statistically not significant (r=0.110, p=0.0887) (Table 2).

When HOMA-IR was categorized into two groups based on a cut-off value of 2.5, the AMS value in the HOMA-IR≥2.5 group was 36.9±12.9 (n=39), which was significantly higher than 30.7±9.0 (n=202) in the HOMA-IR<2.5 group (p=0.0003).

The mean of mental (5 questions), physical (7 questions) and sexual (5 questions) subscale group were 7.56 ± 3.26, 13.5 ± 4.56 and 10.6 ± 3.56, respectively (n=241 in each group). All mental, physical and sexual AMS subscales were significantly positively correlated with FBG, insulin concentrations and HOMA-IR, respectively (Table 2). Only sexual subscale scores were significantly inversely associated with aFT, cFT and cbT but not with TT (Table 2).

AMS 37-49 is defined as moderately severe LOH. We compared the various parameters between subjects with AMS<37 and those with AMS≥37. Table 3 indicates clinical characteristics of total subjects (N=241) and comparison of various values between AMS score <37 group (N=177) and AMS ≥37 group (N=64). Among the various parameters analyzed, including various testosterone values and metabolic parameters, only FBG levels and HOMA-IR displayed significant differences between the two groups. Specifically, both values were higher in subjects with AMS≥37 compared with those in subjects with AMS<37 (Table 3).

Since some association between AMS≥37 and metabolic abnormalities were suggested, we next examined metabolic predictors for AMS≥37. HOMA-IR, with a cut-off value of 2.5, has been generally accepted as a measure of insulin resistance [22]. FBG level of ≥100 mg/dL and HDL-C level of <40 mg/dL consists of IDF criterion of MetS [21]. We previously reported that the serum levels of TT<4 ng/mL or SHBG<47 nmol/L were good and relatively good predictor of MetS, respectively [7]. However, since no cut-off values of adiponectin and CRP for the detection of MetS are available, median values of the serum levels of adiponectin<4.9 μg/mL and CRP≥0.04 were tentatively examined as variables in logistic regression analyses. Univariate analysis revealed that only HOMA-IR≥2.5 was a significant predictor of AMS≥37 (p=0.010). Multivariate analysis demonstrated that adiponectin<4.9 μg/mL and HOMA-IR≥2.5 were significant predictors of AMS≥37 (Table 4).

Fig. 1  Aging Males’ Symptoms (AMS) scores divided by the severity
There were 91 (37.8%), 86 (35.7%), 48 (19.9%) and 16 (6.6%) men with normal (17–26), mild (27–36), moderately severe (37–49) and severe (≥50) AMS scores, respectively and 62.2% displayed abnormal scores (≥27).
Table 2  Correlation between AMS score (total, mental, physical and sexual scores) and various markers

| Factors          | Total       | Mental      | Physical    | Sexual      |
|------------------|-------------|-------------|-------------|-------------|
| Age              | 0.0991      | -0.0329     | 0.0140      | 0.2905 ***  |
| BMI              | 0.0666      | 0.0399      | 0.0481      | 0.0888      |
| Waist C          | 0.1034      | 0.0457      | 0.0984      | 0.1225      |
| TT               | -0.0971     | -0.0186     | -0.1107     | -0.1137     |
| aFT              | -0.0816     | 0.0182      | -0.0529     | -0.1781 **  |
| cFT              | -0.0961     | 0.0072      | -0.0678     | -0.1896 **  |
| cbT              | -0.1119     | 0.0014      | -0.0815     | -0.2109 *** |
| SHBG             | -0.0166     | -0.0162     | -0.0819     | 0.0730      |
| LogCRP           | 0.1099      | 0.1125      | 0.0814      | 0.1325 *    |
| LDL-C            | -0.0311     | -0.0476     | -0.0142     | -0.0256     |
| HDL-C            | -0.0781     | -0.0484     | -0.0416     | -0.1215     |
| TG               | 0.0963      | 0.0261      | 0.1227      | 0.0894      |
| FBG              | 0.1589 *    | 0.1290 *    | 0.1314 *    | 0.1596 *    |
| HbA1c            | 0.1206      | 0.0924      | 0.0853      | 0.1444 *    |
| Insulin          | 0.1839 **   | 0.1684 **   | 0.1475 *    | 0.1729 **   |
| HOMA-IR          | 0.2166 ***  | 0.1966 **   | 0.1769 **   | 0.2013 **   |
| HOMA-β           | 0.1000      | 0.0917      | 0.0764      | 0.0989      |
| Adiponectin      | -0.0380     | -0.0431     | 0.0018      | -0.0695     |

AMS, Aging Males’ Symptoms. AMS scores includes total score and its components (mental, physical and sexual scores). * p<0.05, ** p<0.01, *** p<0.001. BMI, body mass index; Waist C, waist circumference; TT, total testosterone; aFT, free testosterone measured by analogue RIA; cFT, calculated free testosterone; cbT, calculated bioavailable testosterone; SHBG, sex hormone-binding globulin; CRP, c-reactive protein; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; FBG, fasting blood glucose.

Table 3  Clinical characteristics of 241 subjects and comparison of various values between AMS score <37 group (N=177) and AMS score ≥37 group (N=64)

|                     | Total subjects (N = 241) | AMS score <37 (N = 177) | AMS score ≥37 (N = 64) | p values |
|---------------------|--------------------------|--------------------------|-------------------------|----------|
| Age (years)         | 52.7 ± 7.5               | 52.5 ± 8.0               | 53.2 ± 5.8              | 0.511 1) |
| TT (ng/mL)          | 4.90 ± 1.63              | 4.98 ± 1.62              | 4.67 ± 1.63             | 0.196 1) |
| aFT (pg/mL)         | 10.4 ± 3.52              | 10.6 ± 3.50              | 10.1 ± 3.57             | 0.332 1) |
| cFT (pg/mL)         | 81.0 ± 24.5              | 81.5 ± 24.8              | 79.6 ± 23.6             | 0.603 1) |
| cbT (ng/mL)         | 1.89 ± 0.56              | 1.90 ± 0.57              | 1.84 ± 0.55             | 0.466 1) |
| SHBG (nmol/L)       | 47.5 ± 16.5              | 48.2 ± 15.9              | 45.4 ± 17.9             | 0.245 1) |
| CRP (mg/dL)         | 0.04 [0.02-0.08]         | 0.04 [0.02-0.08]         | 0.05 [0.03-0.10]        | 0.081 2) |
| Adiponectin (µg/mL) | 5.55 ± 2.82              | 5.45 ± 2.74              | 5.80 ± 3.00             | 0.398 1) |
| Waist C (cm)        | 86.7 ± 8.02              | 86.4 ± 7.52              | 87.5 ± 9.3              | 0.359 1) |
| HDL-C (mg/dL)       | 54.7 ± 11.6              | 55.4 ± 11.4              | 52.8 ± 11.8             | 0.132 1) |
| TG (mg/dL)          | 127.5 ± 66.8             | 125 ± 63.7               | 135 ± 75                | 0.297 1) |
| FBG (mg/dL)         | 100.2 ± 12.9             | 99.0 ± 9.84              | 103.7 ± 18.6            | 0.013 1) |
| HOMA-IR             | 1.67 ± 1.21              | 1.54 ± 0.97              | 2.03 ± 1.67             | 0.006 1) |
| HOMA-β              | 63.7 ± 32.9              | 62.6 ± 31.3              | 66.6 ± 37.0             | 0.401 1) |

Data are expressed as means ± SD or medians [quartile 25%–75%]. p-values were determined by 1) unpaired t test or 2) Mann-Whitney test. AMS, Heinemann’s Aging Males’ Symptoms; TT, total testosterone; aFT, free testosterone measured by analogue RIA; cFT, calculated free testosterone; cbT, calculated bioavailable testosterone; SHBG, sex hormone-binding globulin; CRP, c-reactive protein; Waist C, waist circumference; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; FBG, fasting blood glucose.
In the present study, 59 men were diagnosed with MetS using IDF criterion (Table 5). As expected, the values of BMI, waist circumference, TG, FBG and hs-CRP in MetS group were significantly higher than those in non-MetS group. The values of HDL-C and adiponectin in MetS group were significantly lower than those in non-MetS group. These results are consistent with the concept of MetS. In addition, the levels of TT, aFT, cFT, cbT, TT and SHBG were significantly lower in the MetS group than those in the non-MetS group, as we previously reported [7]. However, total AMS values were not statistically different between the MetS and non-MetS groups (Table 5). We examined predictive values for MetS detection. By either univariate or multivariate analyses, only TT was a significant predictor of MetS as we previously reported [7]. AMS≥37 was not a significant predictive factor for MetS by either way of analysis (Table 6).

### Discussion

In the present study, we noted that even in relatively healthy male subjects (n=241), a normal AMS score (<26) was observed in only 91 men (37.8%), while abnormal scores (≥27) were observed in the remaining 150 men (62.2%). This finding suggests a higher prevalence of LOH symptoms, even in middle-aged men who did not consult a hospital regarding LOH specifically but participated in a routine examination. Subjective LOH symptoms have been generally accepted to be helpful for androgen deficiency screening [1-3]. However, in our study, no significant association between total AMS scores and testosterone or SHBG levels were observed. Our results together with previous findings [13, 14] suggest that the AMS scale may have a high degree of sensitivity to detect the presence of symptoms/signs of LOH but may be relatively poor in regard to specificity. This is because the detected symptoms/signs can be caused by factors other than testosterone deficiency. Therefore, AMS score alone is not an ideal diagnostic tool for testosterone deficiency and cannot replace analysis of blood testosterone levels [15, 16]. This is why most clinical guidelines of LOH require the questionnaire combined with blood testosterone analysis for diagnosis [2, 3].

The AMS questionnaire includes three subscales, namely psychological, somatic and sexual subscales. However, in a study investigating 81 cases of LOH (53–66 years of age), neither total scores nor individual subscale scores were associated with testosterone levels [12]. In contrast, in our study, sexual subscale scores were significantly and inversely associated with aFT, cFT and cbT levels. Thus, a reduction in free or bioavailable testosterone may not contribute to total AMS values but could contribute to relatively specific LOH symptoms, namely sexual disturbances, in middle-aged men.

Based on our findings, we hypothesized that factors other than androgens might influence AMS values. Our study revealed that AMS values were significantly positively correlated with FBG levels, fasting
Table 5  Testosterone values, AMS scores, CRP concentrations and adiponectin levels in subjects with and without metabolic syndrome based on IDF criterion

|                      | With MetS (N = 59) | Without MetS (N = 182) | p values |
|----------------------|--------------------|------------------------|----------|
| Age (years)          | 52.8 ± 6.7         | 52.6 ± 7.7             | 0.8554   |
| BMI (kg/m²)          | 26.4 ± 3.0         | 23.1 ± 2.4             | <0.0001  |
| Waist C (cm)         | 94.2 ± 7.4         | 84.3 ± 6.6             | <0.0001  |
| TT (ng/mL)           | 4.05 ± 1.38        | 5.17 ± 1.61            | <0.0001  |
| aFT (pg/mL)          | 9.66 ± 3.37        | 10.7 ± 3.53            | 0.0487   |
| cFT (pg/mL)          | 72.3 ± 21.3        | 83.8 ± 24.8            | 0.0017   |
| cbT (ng/mL)          | 1.71 ± 0.50        | 1.94 ± 0.57            | 0.0053   |
| SHBG (nmol/L)        | 40.8 ± 16.0        | 49.6 ± 16.1            | 0.0003   |
| TG (mg/dL)           | 182.3 ± 80.6       | 109.7 ± 50.3           | <0.0001  |
| HDL-C (mg/dL)        | 49.3 ± 10.0        | 56.4 ± 11.5            | <0.0001  |
| FBG (mg/dL)          | 111.5 ± 17.5       | 96.6 ± 8.21            | <0.0001  |
| HOMA-IR              | 2.88 ± 1.66        | 1.28 ± 0.65            | <0.0001  |
| AMS                  | 33.0 [25.0-41.0]   | 28.5 [25.0-36.3]       | 0.1319   |
| hs-CRP (mg/dL)       | 0.06 [0.03-0.11]   | 0.03 [0.02-0.08]       | 0.0014   |
| Adiponectin (µg/mL)  | 4.35 ± 1.43        | 5.93 ± 3.03            | 0.0001   |

Data are expressed as means ± SD or medians [quartile 25%-75%]. The statistical significance of each value was compared between subjects with MetS and those without MetS. p values were determined by 1) unpaired t test or 2) Mann-Whitney test. MetS, metabolic syndrome; TT, total testosterone; aFT, free testosterone measured by analogue RIA; cFT, calculated free testosterone; cbT, calculated bioavailable testosterone; SHBG, sex hormone-binding globulin; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; FBG, fasting blood glucose; AMS, Heinemann’s Aging Males’ Symptoms; CRP, c-reactive protein.

Table 6  Predictors for Metabolic syndrome (IDF criterion) determined by binary logistic regression analysis

|                      | Univariable analysis | Multivariable analysis |
|----------------------|----------------------|------------------------|
|                      | OR (95%CI)           | p values               |
|                      |                      |                         |
| Age (per year)       | 1.00 (0.96 – 1.04)   | 0.883                  |
| TT <4 ng/mL          | 4.86 (2.63 – 8.97)   | <0.001                 |
| aFT <10 pg/mL        | 1.68 (0.93 – 3.00)   | 0.084                  |
| cFT <80 pg/mL        | 2.19 (1.19 – 4.01)   | 0.012                  |
| cbT <1.7 ng/mL       | 1.99 (1.11 – 4.01)   | 0.021                  |
| SHBG <47 nmol/L      | 2.90 (1.56 – 5.39)   | <0.001                 |
| AMS score ≥37        | 1.44 (0.76 – 2.72)   | 0.264                  |
| CRP ≥0.04 mg/dL      | 2.17 (1.21 – 3.92)   | 0.010                  |
| Adiponectin <4.9 µg/mL| 2.26 (1.24 – 4.12)   | 0.008                  |

Univariate analysis was performed before adjustment for other variables. The cut-off value for each variable was the median value. OR, odds ratio; CI, confidential interval; TT, total testosterone; aFT, free testosterone measured by analogue RIA; cFT, calculated free testosterone; cbT, calculated bioavailable testosterone; SHBG, sex hormone-binding globulin; AMS, Heinemann’s Aging Males’ Symptoms; CRP, c-reactive protein.
insulin concentrations and HOMA-IR. Interestingly, FBG, insulin concentrations and HOMA-IR were also inversely associated with psychological, somatic and sexual subscales. In contrast to the relatively specific association between free or bioavailable testosterone and sexual symptoms, insulin resistance may reflect more general unwellness.

Visceral obesity, inflammation and insulin resistance are central to MetS pathogenesis [17, 23]. The findings in our study clearly support the above concept in that hs-CRP values and HOMA-IR were significantly higher in the MetS group than those in the non-MetS group. In addition, levels of the anti-MetS factor, adiponectin, were lower in subjects with MetS than those in subjects without MetS, as previously reported [24, 25]. When we divided patients into two groups using the cut-off value of HOMA-IR 2.5 as a measure of insulin resistance [22], individuals with HOMA-IR≥2.5 displayed significantly higher AMS scores than those with HOMA-IR<2.5, suggesting that AMS values might be increased with insulin resistance. Furthermore, HOMA-IR≥2.5 was a significant predictor of AMS≥37 in both univariate and multivariate analyses. On the other hand, AMS values were not affected by the presence or absence of MetS. Univariate and multivariate binary logistic regression analyses revealed that AMS≥37 was not a significant predictive factor for MetS using IDF criterion. These results suggest that AMS≥37 may reflect general unwellness associated with insulin resistance but is not so strong as a marker for MetS detection compared with established markers, such as serum adiponectin [24, 25] or TT, as we previously reported [7].

Insulin resistance is associated with low-grade inflammation and visceral fat accumulation [17, 26]. Low-grade systemic inflammation is clinically characterized by increased serum levels of hs-CRP, proinflammatory cytokines and chemokine levels [23]. These inflammatory mediators participate in the activation of innate and adaptive immune cells and contribute to tissue damage and insulin resistance [27]. In our study, a weak tendency of positive correlation between log hs-CRP and total AMS scores (p=0.089) was observed. The low-grade physical inflammation even in otherwise healthy subjects might be a background of LOH-like symptoms.

In addition, endogenous testosterone has been suggested to exert its anti-inflammatory effect since increased inflammatory cytokine levels that were suppressed by testosterone administration in hypogonadotropic men have been reported [28]. Haring et al. demonstrated inverse associations between sex hormone concentrations and proinflammatory and oxidative stress markers [29]. In addition, Bobjer J et al. reported that even subnormal testosterone levels at a young age may already associate with low-grade inflammation [30]. Although the association between endogenous testosterone levels and hs-CRP not so clear in our study, only sexual subscale was significantly associated with hs-CRP, suggesting some involvements of low-grade inflammation in sexual disturbance.

There are limitations to our study. Because our study is a cross-sectional study, we cannot definitively conclude that there was a pathogenic relationship between AMS and insulin resistance. The relationship between AMS and insulin resistance was significant but weak. AMS might be affected by other factors that are testosterone-dependent or -independent. Nevertheless, our study suggests some association of AMS values with insulin resistance.

In conclusion, almost two-thirds of middle-aged subjects displayed abnormal AMS scores at a routine health examination, indicating a relatively high background AMS score, even in otherwise healthy individuals. The AMS score was independent of serum testosterone values and displayed a positive correlation with HOMA-IR. Actually, HOMA-IR≥2.5 was a significant predictor for detection of moderately severe AMS values (AMS≥37) by multivariate analysis, reflecting general unwellness caused by insulin resistance.

Acknowledgments

This research was partially supported by a Grant-in-Aid for Scientific Research (B) Japan Society for the Promotion of Science (JSPS) (ID: 23390248). We thank Mrs. K. Kusamoto, M. Ochi, T. Onimaru and R. Makita in the Department of Preventive Medicine of Iizuka Hospital for their help in this study.

Disclosure

The Department of Bioregulatory Science of Life-related Diseases of Fukuoka University (Fukuoka, Japan) was supported financially by a donation from MSD K. K. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of manuscript.
References

1. Namiki M, Akaza H, Shimazu T, Ito N, Iwamoto T, et al. (2008) Clinical practice manual for late-onset hypogonadism syndrome. *Int J Urol* 15: 377-388.

2. Bhasin S, Cunningham GR, Hayes FJ, Matsumoto AM, Snyder PJ, et al. (2010) Testosterone therapy in men with androgen deficiency syndromes: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 95: 2536-2559.

3. Wang C, Nieszlag E, Swerdloff R, Behre HM, Hellstrom WJ, et al. (2009) Investigation, treatment, and monitoring of late-onset hypogonadism in males: ISA, ISSAM, EAU, EAA, and ASA recommendations. *J Androl* 30: 1-9.

4. Rao PM, Kelly DM, Jones TH (2013) Testosterone and insulin resistance in the metabolic syndrome and T2DM in men. *Nat Rev Endocrinol* 9: 479-493.

5. Fan W, Yanase T, Nomura M, Okabe T, Goto K, et al. (2005) Androgen receptor null male mice develop late-onset obesity caused by decreased energy expenditure and lipolytic activity but show normal insulin sensitivity with high adiponectin secretion. *Diabetes* 54: 1000-1008.

6. Chen Y, Copeland WK, Vedanthan R, Grant E, Lee JE, et al. (2013) Association between body mass index and cardiovascular disease mortality in east Asians and south Asians: pooled analysis of prospective data from the Asia Cohort Consortium. *BMJ* 347: f5446.

7. Tanabe M, Akehī Y, Nomiyama T, Murakami J, Yanase T (2015) Total testosterone is the most valuable indicator of metabolic syndrome among various testosterone values in middle-aged Japanese men. *Endocr J* 62: 123-132.

8. Ruige JB, Ouwens DM, Kaufman JM (2013) Beneficial and adverse effects of testosterone on the cardiovascular system in men. *J Clin Endocrinol Metab* 98: 4300-4310.

9. Bianchi VE (2015) Metabolic Syndrome, Obesity Paradox and Testosterone Level. *Endocrinol Metabol Synd* 4: 172.

10. Heinemann LAJ, Zimmermann T, Vermeulen A, Thiel C, Hummel W (1999) A new ‘aging males’ symptoms’ rating scale. *Aging Male* 2: 105-114.

11. Daig I, Heinemann LA, Kim S, Leungwattanakij S, Badia X, et al. (2003) The Aging Males’ Symptoms (AMS) scale: review of its methodological characteristics. *Health Qual Life Outcomes* 1: 77.

12. T’Sjoen G, Feyen E, De Kuyper P, Comhaire F, Kaufman JM (2003) Self-referred patients in an aging male clinic: much more than androgen deficiency alone. *Aging Male* 6: 157-165.

13. Morley JE, Perry HM 3rd, Kevorkian RT, Patrick P (2006) Comparison of screening questionnaires for the diagnosis of hypogonadism. *Maturitas* 53: 424-429.

14. Blumel JE, Chedraui P, Gili SA, Navarro A, Valenzuela K, et al. (2009) Is the Androgen Deficiency of Aging Men (ADAM) questionnaire useful for the screening of partial androgenic deficiency of aging men? *Maturitas* 63: 365-368.

15. Morales A (2014) Testosterone Deficiency Syndrome: an overview with emphasis on the diagnostic conundrum. *Clin Biochem* 47: 960-966.

16. Cabral RD, Busin L, Rosito TE, Koff WJ (2014) Performance of Massachusetts Male Aging Study (MMAS) and androgen deficiency in the aging male (ADAM) questionnaires in the prediction of free testosterone in patients aged 40 years or older treated in outpatient regimen. *Aging Male* 17: 147-154.

17. Festa A, D’Agostino R Jr, Williams K, Karter AJ, Mayer-Davis EJ, et al. (2001) The relation of body fat mass and distribution to markers of chronic inflammation. *Int J Obes Relat Metab Disord* 25: 1407-1415.

18. Giltay EJ, Haider A, Saad F, Grooren LJ (2008) C-reactive protein levels and ageing male symptoms in hypogonadal men treated with testosterone supplementation. *Andrologia* 40: 398-400.

19. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, *et al.* (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28: 412-419.

20. Kuroe A, Fukushima M, Usami M, Ikeda M, Nakai Y, *et al.* (2003) Impaired beta-cell function and insulin sensitivity in Japanese subjects with normal glucose tolerance. *Diabetes Res Clin Pract* 59: 71-77.

21. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, *et al.* (2009) Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation* 120: 1640-1645.

22. Gayoso-Diz P, Otero-Gonzalez A, Rodriguez-Alvarez MX, Gude F, Garcia F, *et al.* (2013) Insulin resistance (HOMA-IR) cut-off values and the metabolic syndrome in a general adult population: effect of gender and age: EPIRCE cross-sectional study. *BMC Endocr Disord* 13: 47.

23. Tracy RP (2003) Inflammation, the metabolic syndrome and cardiovascular risk. *Int J Clin Pract Suppl* (134): 10-17.

24. Matsuzawa Y, Funahashi T, Kihara S, Shimomura I (2004) Adiponectin and metabolic risk. *Arterioscler Thromb Vasc Biol* 24: 29-33.

25. Kaur J (2014) A comprehensive review on metabolic syndrome. *Cardiol Res Pract* 2014: 943162.
26. Alexopoulos N, Katritsis D, Raggi P (2014) Visceral adipose tissue as a source of inflammation and promoter of atherosclerosis. *Atherosclerosis* 233: 104-112.
27. Rotter V, Nagaev I, Smith U (2003) Interleukin-6 (IL-6) induces insulin resistance in 3T3-L1 adipocytes and is, like IL-8 and tumor necrosis factor-alpha, overexpressed in human fat cells from insulin-resistant subjects. *J Biol Chem* 278: 45777-45784.
28. Malkin CJ, Pugh PJ, Jones RD, Kapoor D, Channer KS, et al. (2004) The effect of testosterone replacement on endogenous inflammatory cytokines and lipid profiles in hypogonadal men. *J Clin Endocrinol Metab* 89: 3313-3318.
29. Haring R, Baumeister SE, Volzke H, Dorr M, Kocher T, *et al.* (2012) Prospective inverse associations of sex hormone concentrations in men with biomarkers of inflammation and oxidative stress. *J Androl* 33: 944-950.
30. Bobjer J, Katrinaki M, Tsatsanis C, Lundberg Giwercman Y, Giwercman A (2013) Negative association between testosterone concentration and inflammatory markers in young men: a nested cross-sectional study. *PLoS One* 8: e61466.