Comparison of testosterone fractions between Framingham Heart Study participants and Japanese participants

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Running title

Comparison of testosterone fractions

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Abstract

Objectives: To determine testosterone fractions in Japanese men and to compare the values as stratified by age with those of Framingham Heart Study (FHS) subjects.

Methods: We enrolled 498 healthy Japanese men. Total testosterone (msTT) was assayed by LC-MS/MS, sex hormone-binding globulin (SHBG) was assayed by immunoassay and free testosterone (cFT) was calculated by a lab at the Boston Medical Center. Analog FT (aFT) and total testosterone (iaTT) were determined by immunoassay. We compared msTT and cFT values in the Japanese subjects with values in the American FHS generation 3 cohort.

Results: The mean serum msTT, SHBG and cFT values were 439.4±167 ng/dL, 65.34±30.61 nmol/L and 58.75±20.0 ng/dL, respectively. The correlation coefficients with age for msTT, SHBG and cFT were 0.0010, 0.5041and -0.496, respectively. There were no age-related changes in msTT values in healthy men (P=0.981), while SHBG and cFT levels showed similar age-related changes (P<0.0001). Serum aFT levels (8.24±12.9 pg/mL) showed age-related changes (P<0.0001) regardless of iaTT levels (P=0.828). Serum iaTT values (486.1±1162.5 ng/dL) correlated with serum msTT values

(r=0.740 95%CI, 0.6965 to 0.7781, P<0.0001). Similarly, aFT and cFT values showed a highly significant correlation (r=0.706 95%CI, 0.6587 to 0.7473, P<0.0001). The aFT values were approximately 10% of the cFT values.

Conclusions: In contrast to the FHS cohort, TT values were not associated with advancing age in this Japanese cohort and cannot be used to diagnose late-onset hypogonadism in Japan. The aFT value is a suitable biochemical determinant for diagnosing late-onset hypogonadism syndrome.

Key words

LOH , analog free testosterone , aging , testosterone , SHBG

Abbreviations

aFT=analog-based FT

AMS=Aging Questionnaire

ASA=American Society of Andrology

cFT=calculated FT

CI= Confidence interval

CV= coefficient of variation

DHEA=dehydroepiandrosterone

DHT= dihydrotestosterone

EAA=European Academy of Andrology

EAU=European Academy of Urology

EMAS=the European Male Aging Study

FHS=Framingham Heart Study

FT=free testosterone

iaTT=immunoassay based TT

ISA=International Society of Andrology

ISSAM=The International Society for the Study of the Aging Male

LC-MS/MS =Lliquid chromatography tandem mass mass spectrometry

LOH=Late Onset Hypogonadism

MrOS=Osteoporotic Fractures in Men Study

msTT=LC-MS/MS assay-based TT

RIA=radioimmunoassay

SD=standard deviation

SHBG=Sex hormone-binding globulin

TDS＝testosterone-deficiency syndrome

TT=total testosterone

Introduction

LOH or TDS is a clinical and biochemical syndrome resulting from decreased serum testosterone levels. LOH can negatively impact quality of life and adversely affect multiple organ systems. Accordingly, serum testosterone measurement is the first step in diagnosing LOH and in assessing androgen status.[1,2]

Measurement of TT is generally sufficient for diagnosing androgen excess or deficiency. However, for suspected mild-to-moderate deficiency, measurement of free testosterone is thought to be useful. Currently, several testosterone fractions, including TT, bioavailable testosterone, FT and cFT, can be used to diagnose LOH. Most testosterone is bound to SHBG and to albumin, and, in general, FT is thought to account for only 1–3% of the TT levels. Serum SHBG increases with age and often affects the proportion of testosterone in each fraction. [3]

Although isotope dilution equilibrium dialysis is recommended for accurate FT measurement, this method is time-consuming and is associated with technical difficulties. An alternative is analog immunoassays, which are available for detecting FT levels in clinical settings. However, analog immunoassays have been widely criticized for their lack of accuracy and for the variability of results with fluctuating SHBG concentrations.[4,5] These qualities suggest that the immunoassays do not truly measure FT. A third alternative is to calculate FT using equations based on the law of mass action.[6-8]

Many studies have evaluated age-related changes in serum testosterone fractions in healthy men. However, only two major studies have evaluated the testosterone levels in community-dwelling men in Japan. Iwamoto et al.[9]

reported reference values for serum iaTT and serum aFT, while Okamura et al. [10] analyzed the serum levels of iaTT and cFT using RIA; iaTT in community-dwelling men. Interestingly, neither study found that serum TT declined with age in Japanese men, in contrast with declines in FT with age.

However, other cross-sectional studies did show a decrease in serum TT concentration with age.[11,12] Longitudinal studies, the Massachusetts Male Aging Study[13] and the Baltimore Longitudinal Aging Study[14] all reported decreases in TT with increasing age. Circulating testosterone in men is thought to decline progressively by 0.4–2% per year from the third decade onward.[15] In addition, the ISA, ISSAM, EAU, EAA, and ASA recommend measuring serum TT to establish a diagnosis of hypogonadism.

On the other hand, the FHS is a long-term, ongoing cardiovascular study on residents of the town of Framingham, Massachusetts, USA. The study began in 1948 with 5,209 adult subjects from Framingham, and is now on its third generation (G3) of participants. The FHS participants, and their children and grandchildren, voluntarily consented to undergo a detailed medical history, physical examination, and medical tests every two years, creating a wealth of data about physical and mental health, especially about cardiovascular disease. Hormonal profile is available for this study.

Our study used LC-MS/MS, immunoassay and calculation to determine the testosterone fraction values in Japanese men over 40 years of age. To investigate whether there are ethnic differences in testosterone levels according to age, the data from FHS subjects and Japanese subjects were compared.

Materials and Methods

Patients and samples

This study was approved by the Ethics Committee of the Kanazawa University Graduate School of Medical Science (approval no. 40-H19). This cross-sectional study is a part of the project ”Clinical trial about the utility of the androgen replacement therapy (ART) in late-onset hypogonadism (LOH) syndrome” from 2007 to 2010. This study consisted of screening for healthy men and following intervention study for the candidates.

1682 subjects were enrolled in this screening study and obtained sera from all participants. Of the 1682 subjects, 498 were intentionally selected from three area, because enough of sera in three groups could be sent to the Hormone Assay Laboratory (Boston University School of Medicine, Boston Medical Center, MA, USA) for assay of hormonal parameters.

131, 92 and 275 subjects, which were all ethnically Japanese, were enrolled in Ishikawa (Kanazawa city), Kanagawa (Kamakura city) and Tochigi (Nasushiobara city) prefecture and surrounding area, respectively.

A total of 131 subjects were invited to participate in this study during their regular visits to an urologist and general internist. The volunteers had no history of the presence of any cancers and/or urinary retention due to benign prostatic hyperplasia. Exclusion criteria included cirrhosis or any other liver disease as well as serious psychiatric disorders, use of mood stabilizers, psychotropic and anxiolytic agents as well as medications known to affect the endocrine system and hypothyroidism. Blood samples were obtained between 8:00 and 10:00 AM.

Sera of 92 and 275 subjects, which were hospitalized for a clinical survey to healthy men, were obtained. The blood sample of these 367 subjects were obtained at approximately 8:00 in the morning.

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Total testosterone, free testosterone and SHBG measurements

Blood samples were immediately centrifuged at 4°C. The serum was stored at -80°C until assays were performed. Part of each serum sample was sent to the Hormone Assay Laboratory for TT, SHBG and cFT determinations in March, 2012.

TT was assayed using the msTT. The functional limit of detection, defined as the lowest concentration and detected with less than 20% CV, was 2 ng/dL; no sample was outside the linear range of 2 to 2000 ng/dL. Recovery was calculated by adding known amounts of testosterone to charcoal-stripped serum samples and analyzing the samples by LC-MS/MS. The correlation between the amount added and the amount measured by LC-MS/MS was 0.998. The average recovery was 102±3%. The cross-reactivity of DHEA, DHEAS, DHT, androstenedione and estradiol in the testosterone assay was negligible at ten times the circulating concentrations of these hormones. The interassay coefficient of variation was 15.8% at 12.0 ng/dL, 10.6% at 23.5 ng/dL, 7.9% at 48.6 ng/dL, 7.7% at 241 ng/dL, 4.4% at 532 ng/dL, and 3.3% at 1016 ng/dL.[16] Reference ranges were not indicated in the test report.

FT was calculated using a published law-of-mass-action equation that utilizes an association constant estimated from a systematic review of published binding studies and an iterative numerical method.[17] The intra- and inter-

assay coefficients of variation in the low, medium and high pools were 4.3% and 5.5%, 4.9% and 2.4%, and 8.1% and 2.5%, respectively. Reference ranges were not indicated in the test report.

SHBG levels were measured using a two-site immunofluorometric assay (DELFIA-Wallac, Inc., Turku, Finland).[18,19] The inter-assay CVs were 8.3%, 7.9% and 10.9%, and the intra-assay CVs were 7.3%, 7.1% and 8.7%, respectively, for the low, medium and high pools. The analytical sensitivity of the assays was 0.5 nmol/L. Reference ranges were indicated 12.9-61.7 nmol/L in the test report.

Comparison of total and free testosterone levels with the levels in Framingham Heart Study subjects

This project is based on this longitudinal study. The testosterone levels according to age were compared in Japanese subjects versus FHS subjects; the FHS data is referred in Supplemental Tables 4 and 5 in the literature for comparison.[16] The testosterone determinations were measured using the same assay as in current study.

Analog total and free testosterone measurements

Aliquots of each serum sample were sent to a laboratory (SRL Inc., Tokyo, Japan) for TT and aFT measurement. The iaTT was measured with a commercial chemiluminescent immunoassay (Architect Testosterone kit; Abbott Japan; Tokyo, Japan), and aFT was measured using a kit from Diagnostic Products (Los Angeles, CA, USA). The interassay CV were less than 15% for both kits according to the manufacturer information.

Statistical analysis

Data were analyzed using GraphPad Prism version 5.04 for Windows (GraphPad Software, San Diego, CA, USA; www.graphpad.com). Parameter values are reported as means and SDs. Correlations between the immunoassay and mass spectroscopic values were determined using Pearson correlations. Correlations of serum msTT and serum iaTT or serum aFT and serum cFT were determined using Pearson's simple and partial correlation coefficients. Differences were considered statistically significant at $P < 0.05$.

Results

Subjects

Initially 498 men were enrolled in the study. The mean subject age was 60.5±12.9 years (median 60, range 40–90). The distribution of subjects by age is shown in Figure 1. Of the 498 subjects, the iaTT of 20 could not be assayed as the sample volumes were insufficient. The mean age of the cohort after exclusion of these 20 subjects (n=478) was 60.6±13.0 years (median 59, range 40–90).

Testosterone and SHBG according to age

The mean serum msTT value was 439.4±167 ng/dL (range 139.4–1378), and the median value was 420 ng/dL (Fig. 2a). The mean serum SHBG value was 65.34±30.61 nmol/L (range 10.70–233.8), and the median value was 59.55 nmol/L (Fig. 2b). The mean cFT value was 58.75±20.0 pg/mL (range 16.0–150.8), and the median value was 56.90 pg/mL (Fig. 2c). These three determinations were performed by the Hormone Assay Laboratory at Boston University.

Serum msTT levels showed no decrease with increasing age in healthy men significantly (P=0.981). The correlation coefficient with age for serum msTT level was 0.0010 (95% CI, -0.08684 to 0.08894). In contrast, the correlation coefficients with age for serum SHBG and cFT levels were 0.5041 (95% CI, 0.4355 to 0.5668) and -0.496 (95% CI, -0.5619 to -0.4295), respectively. These correlations were significantly different in age (P<0.0001). Even though the serum msTT level showed no age-related changes, serum SHBG and serum cFT levels showed very similar age-related changes.

Comparison of serum msTT and serum cFT between the Framingham Heart Study and in the current study according to age

Figure 3 shows the relationships of serum msTT and cFT values in the FHS cohort and in the current cohort according to subject age with age stratified by decade. Even though the mean serum msTT did not decline with age in the current cohort, TT was associated inversely with age in the FHS broad sample.[16] The mean serum msTT value in the current samples was approximately 70–80% lower than the mean value in the FHS cohort (Fig.3a). Furthermore, the cFT values decreased with age in both groups. The mean cFT value in the current cohort was approximately 60–70% lower than in the FHS broad sample (Fig.3b).

Comparison of serum iaTT and serum aFT according to age

The mean serum iaTT value was 486.1±1162.5 ng/dL (range 135.0–1100, n=478), and the median value was 472.0 ng/dL (Fig. 2d). The mean serum aFT value was 8.24±12.9 pg/mL (range 21.5–1.90), and the median value was 8.1 pg/mL (Fig. 2e). Serum iaTT levels showed no decrease with increasing age in healthy men significantly (P=0.828). The correlation

coefficient with age for the serum iaTT level was 0.0102 (95% CI, -0.0796 to 0.0998). In contrast, the correlation coefficient with age for serum aFT levels was -0.458 (95% CI, -0.525 to -0.3861). The correlation was significantly different in age (P<0.0001). The serum iaTT level demonstrated no age-related change, while the serum aFT levels in both cohorts demonstrated very similar age-related changes.

Correlation analysis of serum msTT and serum iaTT

Serum iaTT measurements were moderately correlated with the corresponding measurements of serum msTT (Pearson r=0.740 95%CI, 0.6965 to 0.7781, P<0.0001, n=478). The scatter plot in Figure 4a shows the high correlation between analog and LC-MS/MS measurements; the serum iaTT values were higher than the serum msTT values as many samples were over the unity line. The mean serum msTT value (439.4±166.7 ng/dL) was approximately 90% lower than that of serum iaTT (486.1±162.5 ng/dL), and this difference was significant (P<0.0001) (Table 1). Thus, LC-MS/MS results in significantly lower serum testosterone values than those determined by immunoassay; this may be due to substances that interfere with the immunoassay determination.[20 21]

Correlation analysis between serum aFT and serum cFT

Serum aFT values were moderately correlated with cFT values (Pearson r=0.706 95%CI, 0.6587 to 0.7473, P<0.0001, n=498). When using aFT values versus cFT values, it is important to note the strong and highly significant correlation these two assays. As reported previously, the aFT values are lower than the cFT values.[22] In this study, the regression equation was Y = 0.1017X + 2.270. The values for aFT were also lower approximately 10% than

the cFT values (Fig. 4b). As shown in Fig.4b, the slope of regression shifted upper.

Discussion

LOH, also referred to as age-associated TDS, has specific clinical and biochemical features. Accurate testosterone level determination is essential for the diagnosis of LOH. Bhasin et al. determined the reference limits for TT and FT concentrations in a community-based sample of healthy men who were 19–40 years old using data from the FHS third generation (Gen 3) cohort. The FT level was calculated. Values below the 2.5th percentile of the reference sample (n=456) were considered low testosterone values. The 2.5th percentile values were 348.3 ng/dL for TT and 70.0 pg/ml for FT. They demonstrated that values below the proposed lower reference limits were associated with increased risks for conditions that were associated previously with androgen deficiency in one or more cohorts.[18,23]

One large study conducted in 1143 community-dwelling Japanese men aged 20–77 years generated reference testosterone ranges. Specifically, Iwamoto et al. determined serum iaTT and serum aFT levels using RIA (iaTT).[9] In a second study, Okamura et al. analyzed the levels of serum TT and cFT in 1120 community-dwelling Japanese men aged 40–79 years using RIA (iaTT); however, they could not use the term ‘reference ranges’ in their study because they did not follow the formal procedures to determine reference ranges as proposed by the National Committee for Clinical Laboratory Standards. Nonetheless, their serum samples were from community-dwelling men and the determined values were used to evaluate LOH.

Table 1 shows the serum iaTT values in the three studies conducted in Japan. The serum iaTT values did not decrease with age in any of these studies. The mean serum iaTT values according to age by decade in Iwamoto's study were lower than those in the Okamura et al. study and in the current study even though the same assay was used.

Although different commercially available assay kits may give different testosterone values, it appears that the mean serum iaTT value (4.37 ng/mL) is relatively lower in Iwamoto's study, which determined testosterone levels in healthy community-dwelling men. The serum iaTT values in the Iwamoto study and the current study were [mean (+2SD to -2SD)] 4.32 (7.5 to 2.01) ng/mL and (mean±SD) 486.1±162.5 ng/dL (4.86±1.63 ng/mL), respectively. The mean value as determined in the Iwamoto study was relatively low, it is likely to produce bias inclusion of men in their 20s and 30s. In fact we did not measure the values in men in their 20s and 30s, who are likely to have higher testosterone values.

In the current study, the serum iaTT values were higher than the serum msTT values in men aged 40–80+ years. Figure 3a shows the serum msTT in the FHS and current cohorts according to age by decade. The serum msTT value in the current sample was approximately 70–80% lower than that of the FHS broad sample. The distribution of TT levels by decades of age was 10–20% higher in FHS than in the other two cohorts (the MrOS and the EMAS cohorts).[16] A study of an age-stratified, random sample of Rochester (MN, USA) men aged 22 to 93 years included 325 men, with approximately 50 men per decade. The means serum msTT and iaTT values were 467.8±173.4 ng/dL and 492.9±196.2 ng/dL.[24] The TT values in the current cohort is nearly

the same as these in three cohorts. Therefore, it appears likely that the values of msTT in the FHS samples were actually higher contrary to expectations. There is considerable controversy concerning the best method for measuring FT. Although equilibrium dialysis is widely accepted as the ‘‘gold standard’’ for measuring FT,[25] it is considered laborious, slow and costly.[26] There are little laboratories that assay FT using equilibrium dialysis method in Japan. To evaluate FT, the Iwamoto study use an analog FT assay, the Okamura study utilized the equilibrium-binding theory using TT, SHBG and albumin values[6] and the current study utilized the law-of-mass-action equation as described in the Materials and Methods.[17]

Table 2 shows the FT values determined in the three studies conducted in Japan. All three studies found that serum aFT and cFT levels decreased with age (Fig. 2c). The mean serum aFT values according to age by decade in the current cohort were lower than those in the Iwamoto study; both studies used the same detection kit. The mean cFT values according to age by decade in the current cohort were also lower than those in the Iwamoto study, and the mean aFT values according to age by decade in the current cohort were lower than those in the Okamura study. Figure 3b shows that the cFT values in the current cohort were approximately 70–80% lower than in the FHS broad sample. The FT values according to age by decade were 10–20% higher in the FHS cohort than in the other two cohorts (i.e. the MrOS and the EMAS cohorts).[16] Therefore, it is likely that the cFT values in the FHS cohort were higher.

There is general agreement that approximately 44% of the circulating testosterone is strongly bound to SHBG, 54% is loosely bound to albumin and

2% is present as free hormone.[2,27,28] The mean values of serum SHBG according to age by decade in the Current study were very high compared with those determined in subjects in a cross-sectional study of 400 independently-living European and American men between 40 and 80 years of age.[29]

The mean SHBG values by decade of age were 34.7 nmol/L for men in their 40s, 38.0 nmol/L for men in their 50s, 43.6 nmol/L for men in their 60s and 46.1 nmol/L for men in their 70s. The subjects of the Rochester study had a median serum SHBG value of 33.3 nmol/L (quartiles: 24.9–48.3 nmol/L).[24] The mean SHBG values by decade were approximately twice those of the current sample compared with values reported in the literature (Table 2). Biological factors, such as inter-individual variability or ethnic factors, may account for this variation. It is unclear why SHBG levels increase with age, but age-associated decreases in GH and IGF-I levels might contribute to the increase.[30] However, SHBG measurements were strikingly different in different studies, so differences may be due in part to inaccurate measurements.

In conclusion, this large cross-sectional study showed that the TT level did not decrease with age in this Japanese cohort, in contrast to findings in the FHS cohort. Thus, the TT level cannot be used to diagnose LOH in Japan, even though it is recommended for use in European and American populations. However, the aFT can be used as a convenient biochemical determinant for diagnosing LOH syndrome in Japanese men. Future studies should consider that these determinants and SHBG may impact the associations between circulating testosterone fractions and associated clinical conditions.

Furthermore, these determinants could be used to define target populations for male hormone replacement therapy.

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Conflict of interest

None declared.

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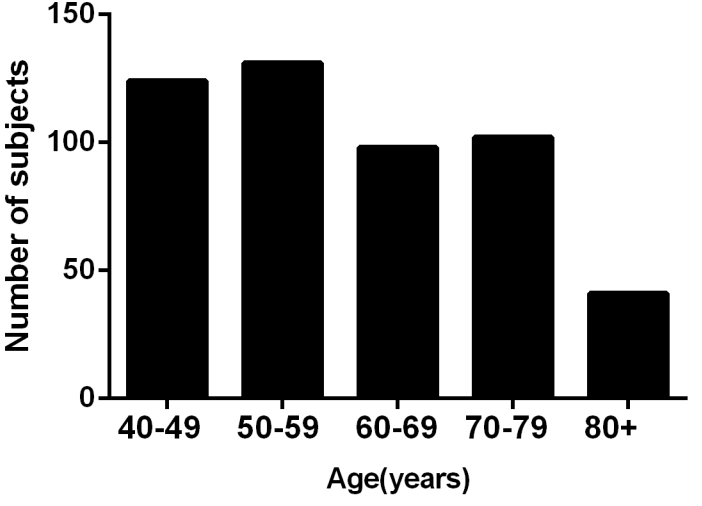
Figure legends

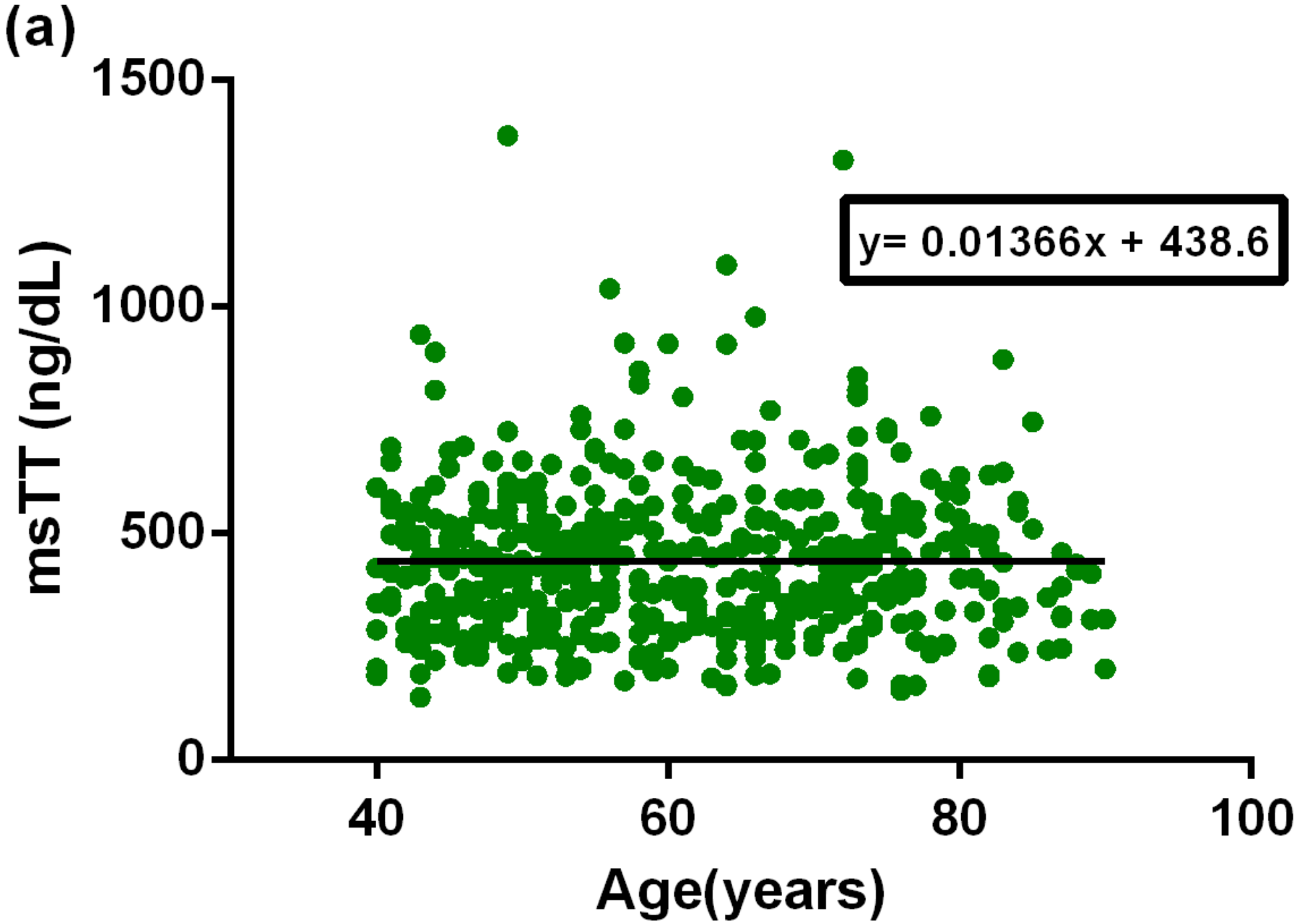
Fig. 1 Distribution of subjects according to age by decade.

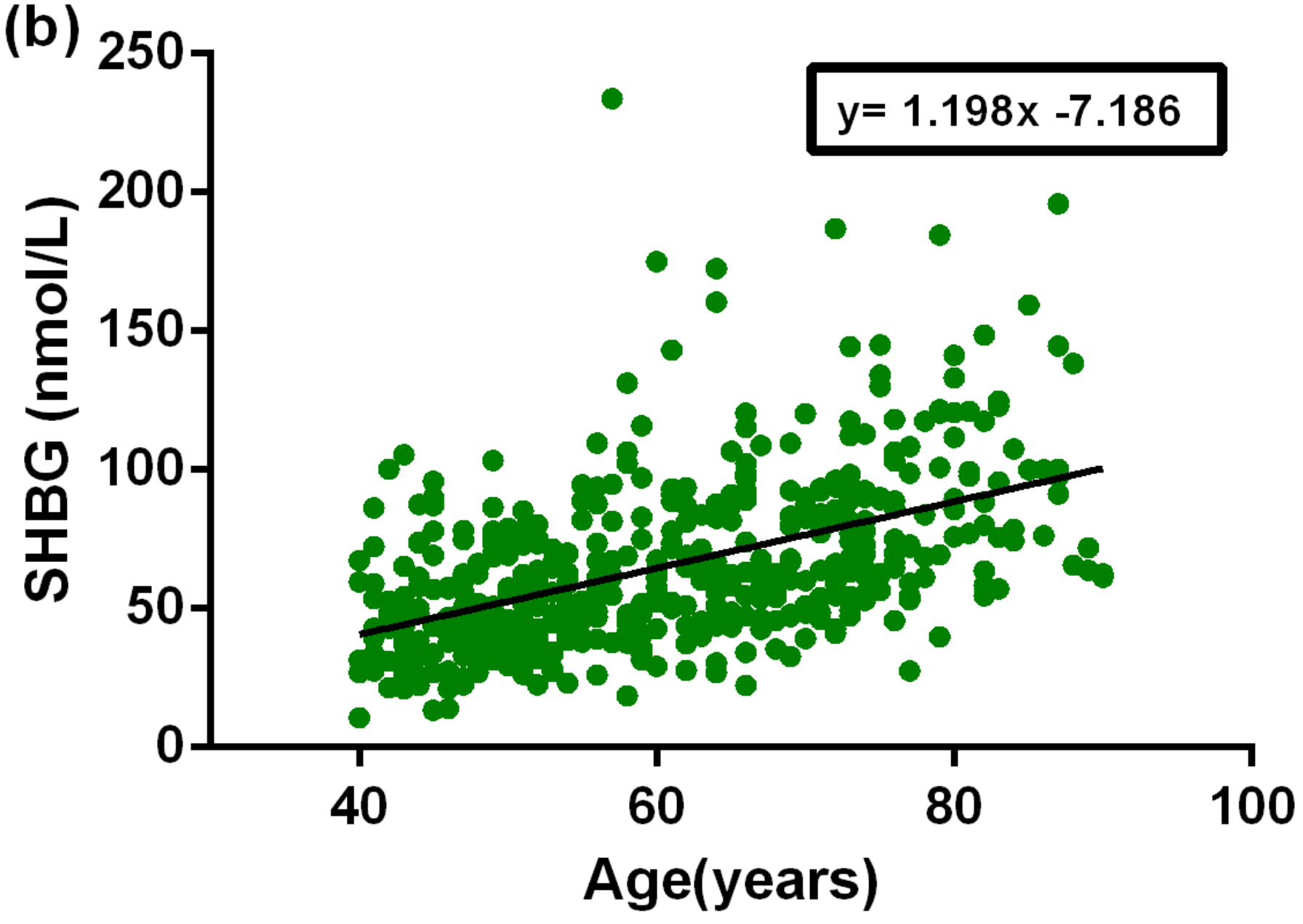
Fig. 2 Scatter blots showing the results of (a) LC-MS/MS analysis of total testosterone (msTT); (b) sex hormone-binding globulin (SHBG); (c) calculated free testosterone (cFT); (d) immunoassay-determined total testosterone (iaTT); (e) and analog-based free testosterone (aFT) according to subject age.

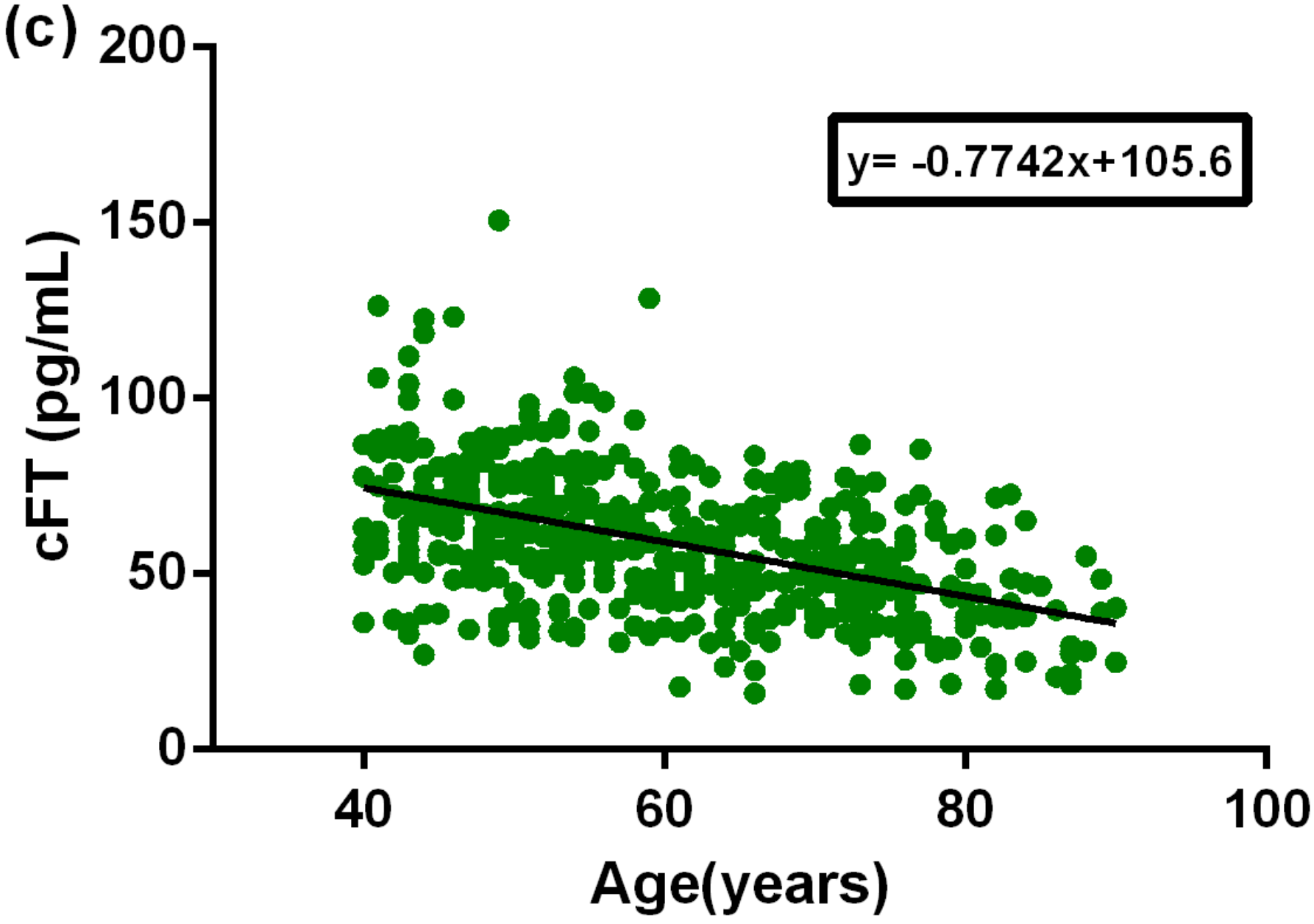
Fig. 3 Comparison of the Framingham Heart Study (FHS) cohort and the Current study cohort using (a) serum LC-MS/MS assay of total testosterone (msTT) and (b) calculated free testosterone (cFT) according to subject age. The filled circles in red and squares in green represent FHS and current subjects, respectively. Vertical lines indicate the standard deviation. Modified from Ref 16.

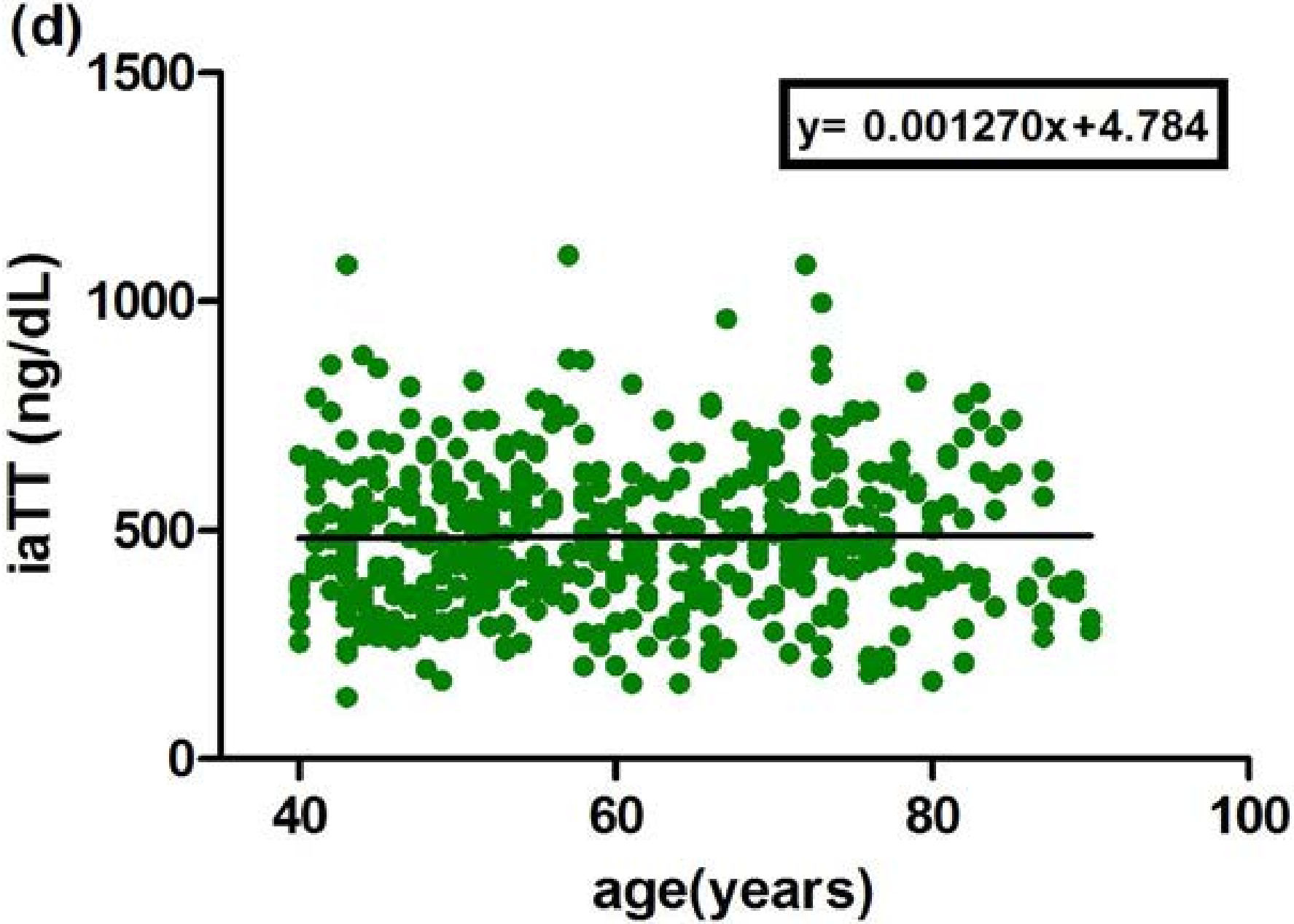
Fig. 4 Correlation analysis. (a) Serum total testosterone determined using immunoassay (iaTT) and using LC-MS/MS assay (msTT). (b) Analog-based free testosterone (aFT) and calculated free testosterone (cFT). In each panel, the solid line indicates the regression relationship and the dashed line represents a slope of unity. The ratio of Y to X is 1.0 (a) and 0.1 (b), respectively.

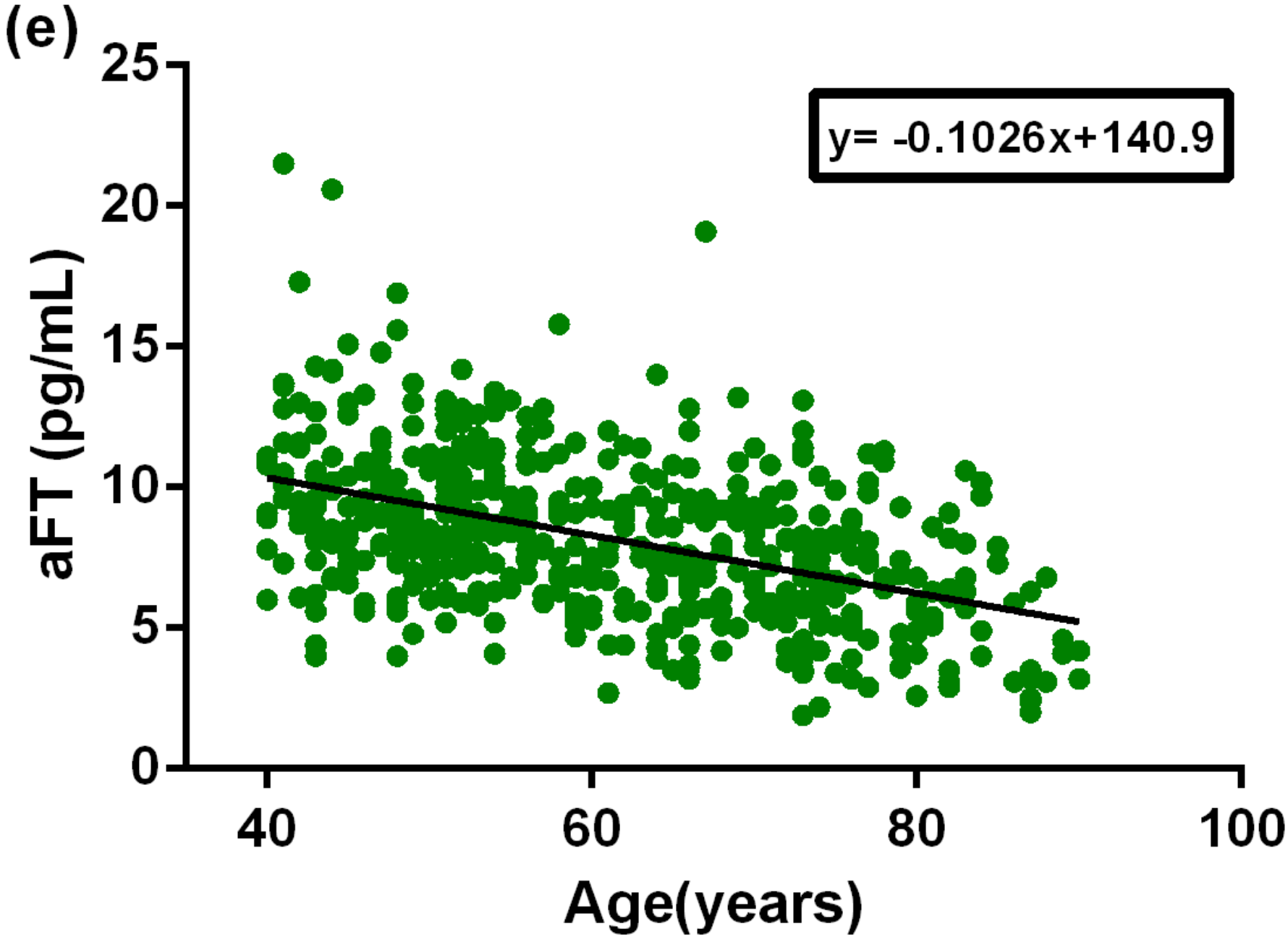

150
100
50
0
Number of subjects
40-49
50-59
60-69
70-79
80+
Age(years)

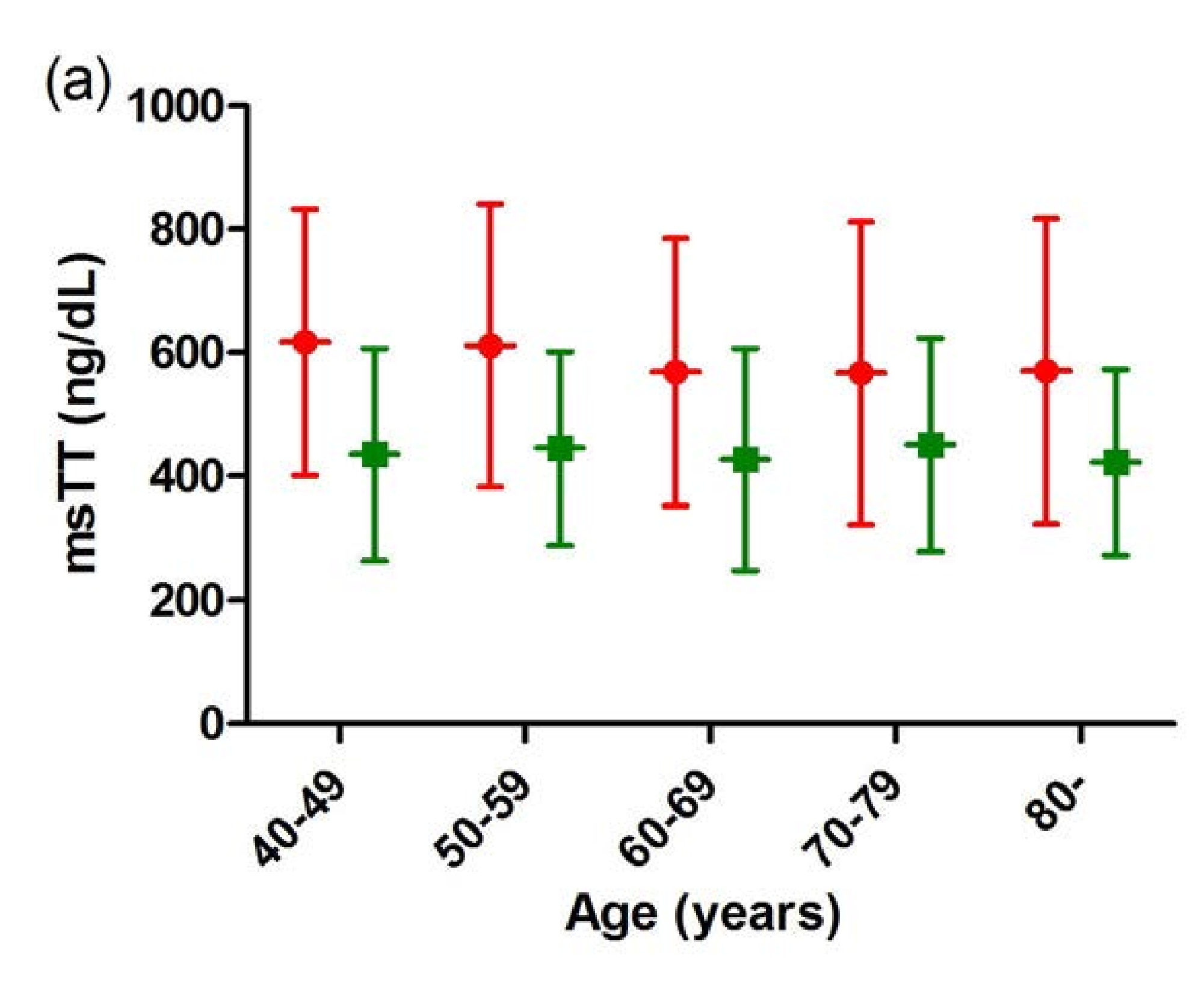

(a)
1500
1000
500
0
msTT (ng/dL)
y= 0.01366x + 438.6
40
60
80
100
Age(years)

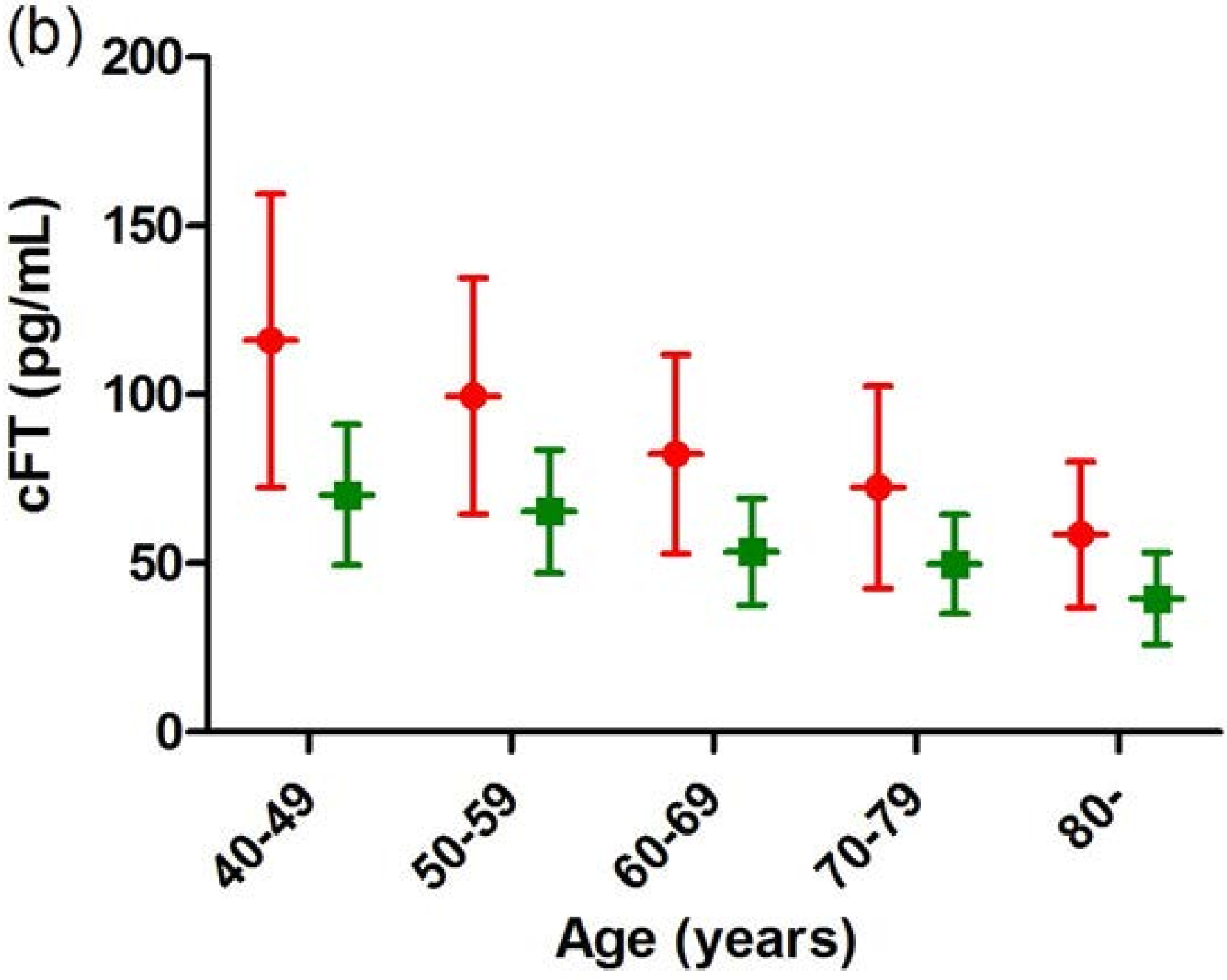

(b)
y= 1.198x -7.186
SHBG (nmol/L)
250
200
150
100
50
0
40
60
80
100
Age(years)


(c)
200
150
100
50
0
cFT (pg/mL)
y= -0.7742x+105.6
40
60
80
100
Age(years)


(d)
1500
1000
500
0
iaTT (ng/dL)
y= 0.001270x+4.784
40
60
80
100
age(years)


(e)
y= -0.1026x+140.9
aFT (pg/mL)
0
5
10
15
20
25
40
60
80
100
Age(years)


(a)
1000
800
600
400
200
0
msTT (ng/dL)
40-49
50-59
60-69
70-79
80-
Age (years)


(b)
200
150
100
50
0
cFT (pg/mL)
40-49
50-59
60-69
70-79
80-
Age (years)

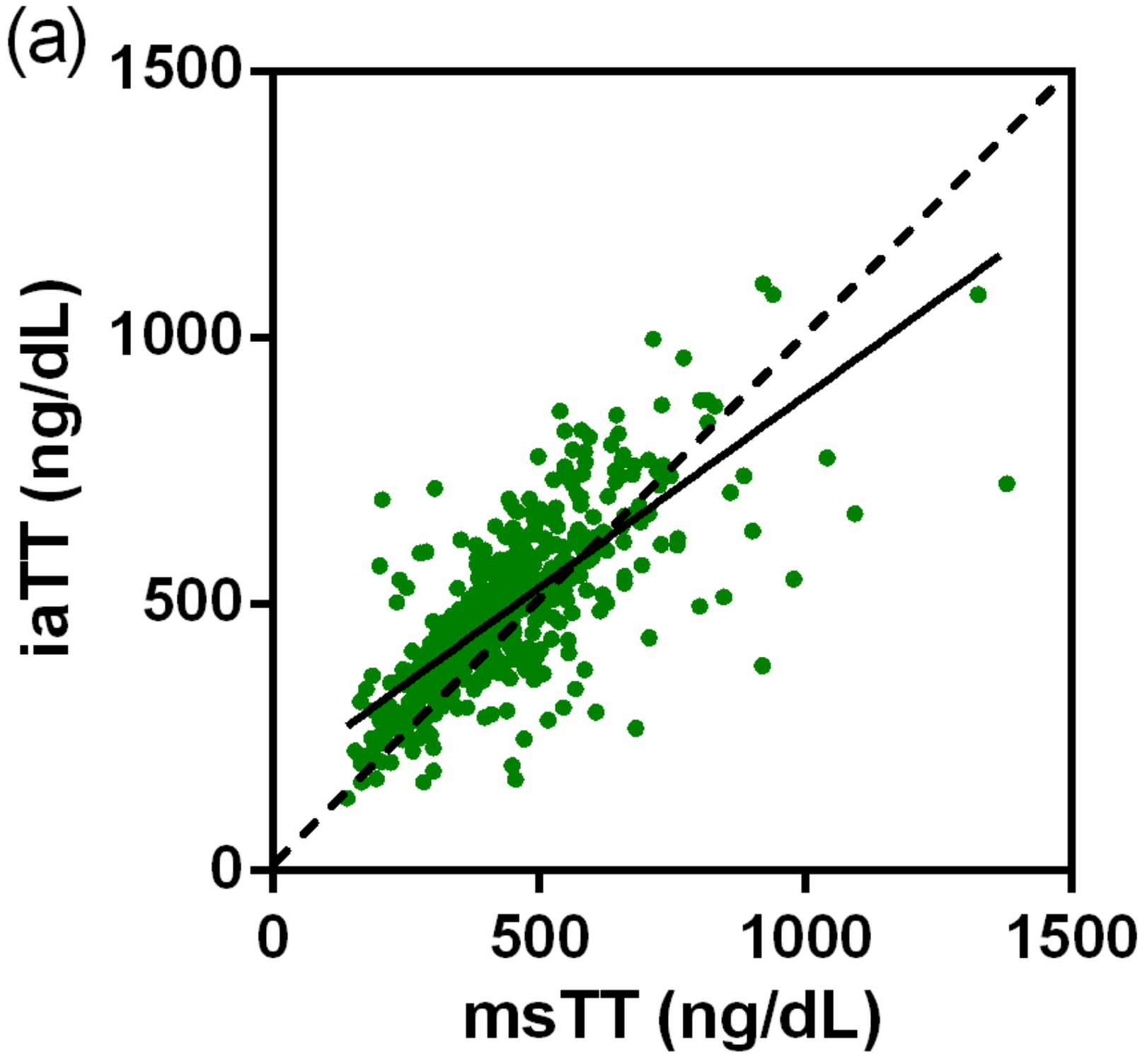


fig4a

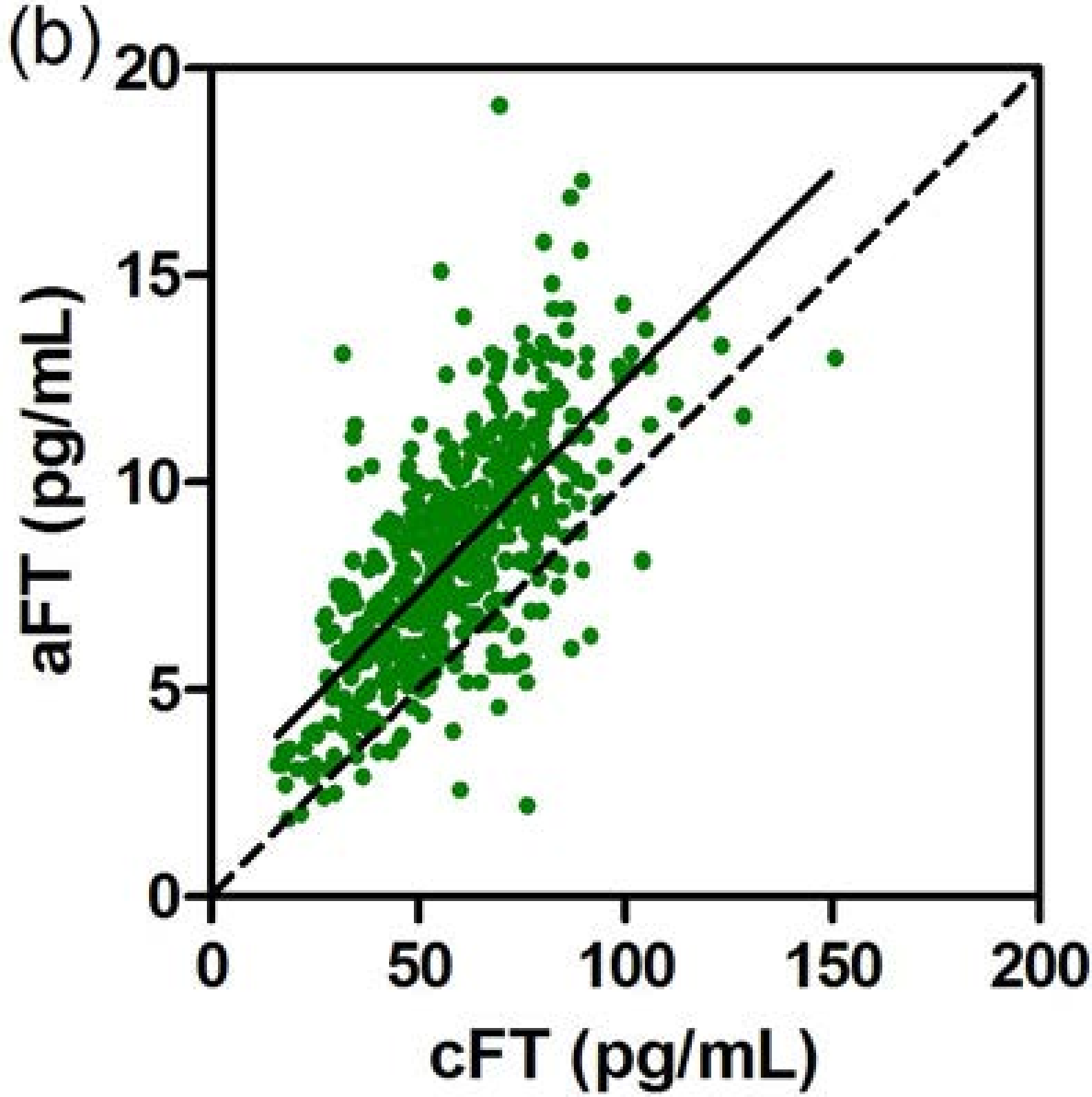

(b)
20
15
10
5
0
aFT (pg/mL)
0
50
100
150
200
cFT (pg/mL)

Table 1. Total testosterone (TT) levels reported in Japanese studies

Age range		20–29	30–39	40–49	50–59	60–69	70–79	80+	Total (n)
Iwamoto et al.									
	iaTT (n)	294	287	235	169	120	38		1143
	mean (+2SD, -2SD) ng/mL	4.98 (8.36, 2.47)	4.3 (7.16, 2.17)	4.2 (7.3, 1.95)	3.91 (6.74, 1.84)	3.83 (6.61, 1.8)	3.84 (6.46, 1.9)	N/A	4.32 (7.5, 2.01)
Okamura et al.									
	iaTT (n)			287	278	276	279		1120
	mean±SD ng/mL	N/A	N/A	5.00±2.94	5.12±3.28	4.99±3.20	5.45±3.56	N/A	5.13±3.26
Current study									
	iaTT (n)			121	125	90	99	43	478
	mean±SD ng/mL	N/A	N/A	4.76±1.68	5.02±1.50	4.66±1.57	5.04±0.17	4.69±1.68	4.86±1.63
	msTT (n)			124	131	98	102	43	498
	mean±SD ng/mL	N/A	N/A	4.34±1.70	4.47±1.53	4.28±1.82	4.54±1.71	4.23±1.50	4.39±1.67

iaTT=immunoassay based TT, msTT=LC-MS/MS assay-based TT, N/A=not available

Table 2. Free testosterone(FT) level and SHBG reported in Japanese studies

Age range		20–29	30–39	40–49	50–59	60–69	70–79	80+	Total (n)
Iwamoto et al.									
	aFT (n)	294	287	235	169	120	38		1143
	mean (+2SD, -2SD) pg/mL	16.8 (27.9, 8.5)	14.3 (23.1, 7.6)	13.7 (21.6, 7.7)	12.0 (18.4, 6.9)	10.3 (16.7, 5.4)	8.5 (13.8, 4.5)	N/A	8.5 (13.8, 4.5)
Okamura et al.									
	aFT (n)			127	121	102	121		471
	mean±SD pg/ml	N/A	N/A	15.1±8.4	13.9±6.8	12.0±6.6	11.5±7.0	N/A	13.2±7.8
	cFT (n)			127	121	102	121		471
	mean±SD pg/ml	N/A	N/A	88.2±43.8	82.3±35.8	70.9±38.8	65.0±38.8	N/A	77.0±43.4
Current study									
	aFT (n)			121	125	90	99	43	478
	mean±SD pg/ml	N/A	N/A	9.74±2.29	9.00±2.29	7.89±2.69	7.00±2.35	5.48±2.24	8.25±2.89
	cFT (n)			121	125	90	99	43	478
	mean±SD pg/ml	N/A	N/A	70.2±20.1	65.4±18.2	53.4±15.9	49.7±14.7	39.6±13.6	58.8±1.63
	SHBG (n)			121	125	90	99	43	478
	mean±SD nmol/L	N/A	N/A	48.4±20.2	56.2±26.4	68.0±28.8	80.3±28.6	97.9±31.5	65.2±30.5

aFT=analog free testosterone, cFT=calculated free testosterone, SHBG= Sex hormone-binding globulin, N/A=not available