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Normal Values of Circulating IGF-I Bioactivity in the Healthy Population: Comparison with five widely used IGF-I immunoassays

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Abstract

Background: IGF-I immunoassays are primarily used to estimate IGF-I bioactivity. Recently, an IGF-I specific Kinase Receptor Activation Assay (KIRA) has been developed as an alternative method. However, no normative values have been established for the IGF-I KIRA.

***Objective*:** To establish normative values for the IGF-I KIRA in healthy adults.

***Design*:** Cross-sectional study in healthy non-fasting blood donors.

***Study participants*:** 426 healthy individuals (310 M, 116 F; age range: 18 – 79 yrs)

***Main outcome Measures*:** IGF-I bioactivity determined by the KIRA. Results were compared with total IGF-I, measured by five different IGF-I immunoassays.

***Results*:** Mean (± SD) IGF-I bioactivity was 423 (± 131) pmol/L and decreased with age (β = -3.4 pmol/L/yr, $p < 0.001$). In subjects younger than 55 yrs mean IGF-I bioactivity was significantly higher in women than in men. Above this age this relationship was inverse, suggesting a drop in IGF-I bioactivity after menopause. This drop was not reflected in total IGF-I levels. IGF-I bioactivity was significantly related to total IGF-I (r_s varied between 0.46 – 0.52; P-values < 0.001).

***Conclusions*:** We established age-specific normative values for the IGF-I KIRA. We observed a significant drop in IGF-I bioactivity in women between 50 and 60 years, which was not perceived by IGF-I immunoassays. The IGF-I KIRA, when compared to IGF-I immunoassays, theoretically has the advantage that it measures net effects of IGF-binding proteins on IGF-I receptor activation. However, it has to be proven whether information obtained by the IGF-I KIRA is clinically more relevant than measurements obtained by IGF-I immunoassays.

Introduction

Fifty years ago Salmon and Daughaday discovered a factor in serum which stimulated sulphate incorporation by cartilage in vitro (1). This unknown factor, then called sulphation factor or somatomedin-C, was later renamed insulin-like growth factor-I (IGF-I) (2, 3). After generation of highly specific antibodies for IGF-I, it became possible to develop immunoassays for the assessment of circulating IGF-I levels (4-6). Today, these IGF-I immunoassays are clinically widely used to assess IGF-I bioactivity in humans and are applicable to measure large numbers of blood samples.

In the circulation about 99% of circulating IGF-I is bound to six high affinity IGF-binding proteins (IGFBPs) (7). IGFBPs interfere with antibody binding to IGF-I and therefore, in virtually all common IGF-I immunoassays an extraction method has to be used in order to remove these IGFBPs (8, 9). Remaining IGFBPs or their fragments may interfere and produce falsely increased or decreased circulating total IGF-I levels (10). This latter phenomenon may especially occur in pathological conditions (11).

Another problem is that large differences in absolute circulating levels of total IGF-I are observed between different commercially available IGF-I immunoassays (8, 12). Recently it was suggested that this variability in assay performance and the use of inappropriate reference ranges undermine the applicability of international consensus criteria in local practice (11). Nevertheless, introduction of IGF-I immunoassays has been proven to be useful in the diagnosis and treatment of acromegaly (13).

The IGFBPs are considered to regulate IGF-I bioavailability (7, 14). However, the commonly used IGF-I immunoassays in fact ignore the effects of IGFBPs on the interactions between IGF-I and the IGF-I receptor (10). Frystyk et al. recently developed a kinase receptor activation assay (KIRA) specific for IGF-I (15, 16). This IGF-I KIRA quantifies phosphorylation of tyrosine residues of the activated IGF-I receptor (IGF-IR) as a measure for IGF-I bioactivity in serum (17). In contrast to commonly used IGF-I immunoassays, the IGF-I KIRA is sensitive for modifications of IGF-IR activation by circulating IGFBPs and IGFBP-proteases (15, 18). Therefore, the IGF-I KIRA method might be an important advancement in measuring circulating IGF-I bioactivity, which could enhance insights in the IGF-I system both in normal and pathological conditions.

The aim of the present study was to establish normative values for the IGF-I KIRA in the healthy population. Results of the IGF-I KIRA were compared with circulating total IGF-I levels obtained by five commonly used IGF-I immunoassays (19).

Research Design and Methods

Subjects and measurements

The study population has been described previously (19). Briefly, morning serum samples were taken from healthy non-fasting blood donors (N = 426; females N = 116). Age ranged from 18 to 79 years (median: 44 yrs). Height and weight were measured and the body mass index (BMI) was calculated. Mean ± SD for BMI was 25.3 ± 3.9 kg/m^2 (range: 15.8 – 42.2). All participants gave informed consent. The Ethics Committee of the Charité Humboldt University (Berlin, Germany) had approved this study.

Total IGF-I Immunoassays

Five different immunoassays were used to measure total circulating IGF-I in the healthy population. Three of these assays were immunometric assays, whereas two were conventional radioimmunoassays (RIAs). The following immunoassays were used: Assay A: IGF-I RIA, an in-house assay at University Children's Hospital, Tuebingen, Germany; Assay B: IGF-I RIA-CT™, Mediagnost, Tuebingen, Germany; Assay C: Diagnostic Systems Laboratories (DSL) 2800 Active® IGF-I-IRMA, Sinsheim, Germany; Assay D: Nichols Advantage® Chemiluminiscence IGF-I Immunometric assay, Nichols Institute Diagnostics, San Juan Capistrano, CA, USA.; Assay E: IGF-I CIA, Immulite®, Diagnostic Products Corp. (DPC). In all immunoassays recombinant human IGF-I was used as standard. After acidification an excess of IGF-II was used to eliminate residual interference with IGFBPs. For four of these immunoassays

(Assays 1, 2, 3 and 4), the age-related reference ranges for circulating total IGF-I in this study population have been previously published (19). Intra- (a) and inter- (b) assay coefficients of variation varied between (a) 3.1 – 7.0% and (b) 3.8 – 8.8% respectively (19). Total IGF-I levels are expressed as nmol/L (to convert total IGF-I levels into μg/L, values have to be divided by 0.131).

IGF-I Kinase receptor activation assay (IGF-I KIRA)

Circulating IGF-I bioactivity was measured using an in-house IGF-I kinase receptor activation assay as was previously described (15). This assay uses human embryonic renal cells stably transfected with cDNA of the human IGF-IR gene (293 EBNA IGF-IR). Cells were a kind gift from Prof. Pierre de Meyts, (Receptor Biology Laboratory, Hagedorn Research Institute, Novo Nordisk, Gentofte, Denmark). After 48 ours of culture, cells were stimulated at 37°C with either recombinant IGF-I standards (Austral Biologicals, San Ramon, CA) or 10-fold diluted serum samples for 15 minutes and lysed afterwards. Crude lysates were transferred to a sandwich assay. For capture a monoclonal antibody directed against the human IGF-IR (MAD1, 1μg/well, Gropep, Adelaide, Australia) was used. As tracer a europium-labelled monoclonal anti-phosphotyrosine antibody (PY20, Perkin-Elmer Life Sciences) was used. Contents were read in a time-resolved fluorometer (Victor^2 multilabel counter, Perkin-Elmer, Groningen, The Netherlands). Assays were performed in 48 well plates. IGF-I standards, 2 control samples, and unknown serum samples were included in duplicate on every plate. Intra-assay CV was 5.6%. The inter-assay CVs were respectively 6.8% and 12.6% for the two control samples, which averaged (mean ± SD) 414 ± 28 pmol/L and 1146 ± 144 pmol/L (N = 60 plates), respectively. Circulating IGF-I bioactivity is expressed as pmol/L (to convert IGF-I bioactivity into μg/L, values have to be divided by 131). Serum samples used in the IGF-I KIRA were kept at -80°C and had been thawed ones. From previous unpublished data we know that repetitive freezing and thawing of serum samples (up to several times) does not change results of the IGF-I KIRA. IGF-I KIRA measurements were performed 5 years after initial collection of serum.

Statistical Analysis

Data were analyzed using SPSS for Windows, release 12.0 (SPSS, Chicago, Illinois) unless otherwise reported. For IGF-I bioactivity measurements means ± SD, medians, and the 95% confidence intervals (95% CI) are presented. The Kolmogorov-Smirnov test with the Dallal-Wilkinson-Lilliefors correction (K-S test) and the D′Agustino and Pearson omnibus test (A-P test: GraphPad version 5.0, GraphPad Software, Inc., San Diego) were used to test data for normality of distribution. When no normality of distribution was found, data were log-transformed. Correlations between IGF-I bioactivity and total IGF-I are presented as Spearman correlation coefficients (r_s). Nonparametric Mann-Whitney or Wilcoxon rank sum tests were used to compare IGF-I levels between men and women categorized by age. Linear regression was used to calculate the relationship between IGF-I bioactivity and age. The coefficient of variation (CV) was calculated by using the formula: (SD/mean) ×100%. This CV standardizes the relative spread in data between IGF-I assays, so that a sensible comparison can be made. Curve estimation and regression analysis were performed to determine whether age-related changes were best fitted by a linear, exponential or polynomial function. Where more than one function was significant, the one with the highest R^2 value was considered the best fitting model. A P value < 0.05 was considered statistically significant.

Results

Circulating IGF-I bioactivity was almost normally distributed (untransformed data: K-S test; p = 0.07, A-P test; p = 0.04, (Figure 1A)). Log-transformation of IGF-I bioactivity levels did not improve normality of the data distribution (log-transformed data: K-S test; p = 0.0001, A-P test; p < 0.0001). In contrast, circulating total IGF-I levels showed an asymmetric distribution in all five studied IGF-I immunoassays (untransformed data: K-S and

A-P tests; $p < 0.0001$ for all immunoassays (Figure 1B – 1F)). After log-transformation of total IGF-I levels a normal distribution (K-S and A-P test: $p > 0.05$) was obtained in three out of five IGF-I immunoassays (data not shown). (Total IGF-I assays that did not show a normal distribution after log transformation were: DSL IGF-I IRMA (K-S test; $p = 0.003$, A-P test; $p = 0.005$) and Nichols IGF-I CIA (K-S test; $p = 0.03$ and A-P test; $p = 0.02$). Linear correlation and regression coefficients between the measurements of IGF-I immunoassays are shown in Table 1.

To estimate the spread in the data of IGF-I in the study population, we calculated the coefficient of variation (CV). The CV for the IGF-I KIRA was 31% and thereby lower than for the five studied IGF-I immunoassays (Mediagnost IGF-I RIA: 34%, In-house IGF-I RIA: 36%, DPC IGF-I CIA: 42%, Nichols IGF-I CIA: 45%, DSL IGF-I IRMA: 48%, respectively).

Mean (± SD) circulating IGF-I bioactivity was 423 (± 131) pmol/L and ranged from 57 to 875 pmol/L. In Table 2 mean values of circulating IGF-I bioactivity are presented after stratification for age. IGF-I bioactivity decreased significantly with age, which was best fitted by a linear model (slope $\beta = -3.4$ pmol/L/yr, (95% CI: -4.5 – -2.5); $p < 0.001$). There was no significant difference in β ($p = 0.16$) between men ($\beta = -2.9$ pmol/L/yr (95% CI: -3.8 – -1.9); $p < 0.0001$) and women ($\beta = -4.6$ pmol/L/yr (95% CI: -6.0 – -3.3); $p < 0.001$) (Figure 2)). With one exception (Nichols), the age-related decreases in total IGF-I were best fitted by polynomial (quadratic) functions (data not shown). The decrease of total IGF-I with age measured by the total IGF-I CIA (Nichols) was best fitted by a linear model ($\beta = -0.48$ nmol/L/yr, (95% CI: -0.54 – -0.42); $p < 0.001$).

Overall, there were no gender specific differences in mean IGF-I bioactivity (men: mean = 420 pmol/L, (95% CI: 405 – 435) *vs.* women: mean = 433 (95% CI: 409 – 458); $p = 0.36$ (adjusted for age and BMI)).

In subjects younger than 55 yrs, mean IGF-I bioactivity was significantly higher in women than in men (men (N = 207) mean = 436 pmol/L, (95% CI: 418 – 454) *vs.* (women (N = 76): mean = 484 (95% CI: 455 – 513); $p = 0.007$, (Figure 3)). Above the age of 55 years this relationship was opposite and mean IGF-I bioactivity in women was significantly lower than in men (men (N = 103) mean = 387 pmol/L, (95% CI: 366 – 408) *vs.* women (N = 40): mean 337 (95% CI: 313 – 361); $p = 0.008$). In all five IGF-I immunoassays there were no gender-specific differences in mean circulating total IGF-I levels before age 55 yrs (data not shown). Above age 55 years mean circulating total IGF-I levels were significantly lower in women than in men in all IGF-I immunoassays (data not shown). The statistically significant drop in IGF-I bioactivity in women around age 55 years was not observed for all five IGF-I immunoassays.

Circulating IGF-I bioactivity was significantly related to circulating total IGF-I levels (r_s varied between 0.46 – 0.52, $p < 0.001$ for all five IGF-I immunoassays, Figure 4A – 4E). Mean circulating IGF-I bioactivity calculated as percentage of total IGF-I averaged 1.8 to 2.4% (in-house RIA: 2.4% (0.42 – 5.82) (median (range)), Mediagnost: 2.2% (0.34 – 5.2), DSL: 2.3% (0.33 – 9.1), Nichols: 1.8% (0.32 – 5.88) and DPC: 1.8% (0.29 – 5.38). This calculated bioactive IGF-I fraction increased significantly with age for all five IGF-I immunoassays (β varied between 0.010% – 0.033% per year; $p < 0.001$ for all). IGF-I bioactivity was not related to height, weight or BMI, whereas total IGF-I levels were positively related to height (r varied between 0.17 – 0.23, $p < 0.003$ for all), and negatively to BMI (r_s varied between - 0.24 and -0.20, $p < 0.001$ for all).

Discussion

To our knowledge this is the first study that reports age-specific normative ranges of circulating IGF-I bioactivity in the healthy population obtained by the IGF-I KIRA. These normative values are based on measurements in more than 400 normal subjects with age ranging from 18 – 79 yrs. These age-specific normative ranges will be helpful to interpret whether IGF-I bioactivity is normal, increased and/or decreased in subjects with pathological conditions like acromegaly and GH deficiency.

Currently used IGF-I immunoassays have indeed yielded important and biologically meaningful information about the IGF-I system (20). However, many problems have been reported when IGF-I immunoassays were

used in clinical practice (13). Attempts to resolve these problems have focused on methods of separating IGF-I from its binding proteins (IGFBPs) prior to IGF-I measurements. Although there have been many technologies developed to eliminate interference of IGFBPs, in many IGF-I immunoassays, remaining IGFBPs or BP fragments may still interfere and produce falsely increased or decreased circulating total IGF-I levels (21). This latter effect may especially be of importance in pathophysiologic states accompanied by significant changes in IGFBP levels, such as diabetes mellitus and renal failure (21). For example, Chestnut and Quarmby showed that while the correlation between IGF-I immunoassays was high in sera of healthy individuals, there was a lack of correlation between immunoassays when sera from individuals with diabetes were analysed (8).

IGF-I immunoassays only determine the immunoreactive properties of the IGF-I molecule, rather than its actual biological effect (22). The separation of IGF-I from the IGFBPs prior to the IGF-I measurements ignores in fact the important modulating effects of IGFBPs on IGF-I bioavailability. However, clinicians are generally interested in the biological effects of IGF-I (22).

An important reason for using IGF-I immunoassays has been the lack of reliable IGF-I bioassays (10, 16). Previous IGF-I bioassays were based on downstream signalling events (e.g. sulphate incorporation by cartilage, cell proliferation and survival), but they often suffered from high variability and long assay duration (16, 23, 24). Moreover, these IGF-I bioassays often had a lack of specificity and were labour-intensive. An ideal assay for assessing IGF-I bioactivity should be easily quantifiable, highly sensitive and based on a signal specifically transmitted by the IGF-IR (25). In this respect, the IGF-I KIRA comes close to an ideal IGF-I bioassay as it directly targets the activated IGF-IR, requires only small volumes of serum, has a short incubation time, is sensitive to the modifying influences of circulating IGFBPs and IGFBP-proteases and has an overall precision that is fully comparable to the traditional IGF-I immunoassays (15, 16, 18). However, the KIRA is still more labour-intensive than immunoassays. In addition, we realize that IGF-I KIRA measurements were performed with serum and therefore obtained results do not necessarily reflect net IGF-I bioactivity present in the extra-vascular tissues.

Interestingly, IGF-I immunoassays that did not utilize removal of IGFBPs have been described previously in literature (5). These older assays for IGF-I were not considered useful for clinical practice, as it was assumed that IGFBP interference was a priori bad for determination of IGF-I (9). However, this opens the possibility that the results of these older IGF-I assays might correlate better with IGF-I KIRA results than do modern IGF-I immunoassays, which prior to its measurement extract IGF-I from IGFBPs.

Circulating IGF-I bioactivity showed a wide inter-individual variability among subjects in every age group. The CV of the IGF-I KIRA within the study population (a standardized measure of relative spread in data) was lower than that for total IGF-I. Since a lower magnitude of CV is considered to reflect a better reliability (precision) of measurements, this suggests that the IGF-I KIRA in this respect performs at least equal to IGF-I immunoassays.

IGF-I bioactivity was positively related to total IGF-I in all studied IGF-I immunoassays. Interestingly, for all IGF-I immunoassays the observed correlation coefficients were relatively low and comparable. Our results show that the observed relation between total IGF-I and IGF-I bioactivity is independent of the type of immunoassay that is used to determine circulating total IGF-I levels. In addition, these results suggest that the IGF-I KIRA produces new information about the IGF-I system, which differs from that obtained by IGF-I immunoassays. However, the physiological importance of this difference remains to be clarified.

Circulating IGF-I bioactivity decreased significantly with age. The decline of IGF-I bioactivity with age was less steep than that observed for circulating total IGF-I levels. As a consequence the mean calculated fraction of IGF-I bioactivity over total IGF-I increased slightly but significantly with age. Although the cross-sectional study design does not reflect the intra-individual rate of change of IGF-I bioactivity, a possible explanation for

this latter observation is that IGF-I bioactivity becomes less-GH dependent with aging than total IGF-I levels. Another explanation could be that the relative increase in IGF-I bioactivity with age reflects a compensatory mechanism to overcome an age-dependent relative IGF-IR resistance. Third, also circulating IGF-II levels could be involved. In contrast to IGF-I, circulating levels of IGF-II do not decrease after puberty but remain stable throughout life (26). Chen et al. showed that the 293 EBNA IGF-IR cells respond not only to IGF-I but also to IGF-II, which has a cross-reactivity of 12%, to the IGF-I KIRA compared to IGF-I (15). This opens the possibility that the relative contribution of IGF-II to the IGF-I KIRA signal increases with aging.

Remarkably, before age 55 years of age, the mean IGF-I bioactivity was significantly higher in women than in men, while after this age an inverse relationship was observed. This significant drop in IGF-I bioactivity in women between the age of 50 and 60 years was not reflected in total IGF-I levels. Although we do not have information about age of menopause in the studied women, we speculate that the observed decrease in IGF-I bioactivity after age 55 years of age is related to changes in estrogens levels (27-29). This could explain why women had a relatively higher mean IGF-I bioactivity than men before 55 years, but also why mean IGF-I bioactivity was lower in women than in men after this age.

In conclusion, in the present study we established age-specific normative values for circulating IGF-I bioactivity levels in the healthy adult population. The IGF-I KIRA produces new information about the circulating IGF-I system that differs from that obtained by IGF-I immunoassays. Whether this information is clinically more relevant than measurement of circulating total IGF-I levels in the diagnosis and/or treatment of GH disorders is at present unclear. However, the establishment of these normative reference values for IGF-I bioactivity is the first step to answer these questions in the near future.

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Figure legends

Figure 1: Distribution of IGF-I measurements in the study population: **A)** IGF-I KIRA; **B)** IGF-I RIA, an in-house assay at University Children's Hospital, Tuebingen, Germany; **C)** IGF-I RIA-CT™, Mediagnost; **D)** IGF-I-IRMA 2800 Active®, DSL; **E)** IGF-I CIA, Nichols Advantage®; **F)** IGF-I CIA, Immulite® DPC. Data obtained by the IGF-I KIRA showed an almost normal distribution, in contrast to IGF-I immunoassays for which data were skewed leftwards. An ideal bell-shaped normal distribution curve is shown in each plot.

Figure.2: IGF-I bioactivity levels according to age. IGF-I bioactivity decreased significantly with age in both men (□) and women (●). Linear regression lines for men (▬) and women (▪▪▪) are shown.

Figure 3: Mean circulating IGF-I bioactivity according to age categories and sex. Mean circulating IGF-I bioactivity levels in women (▪▪▪) differed significantly from men (▬), being higher in age groups 35-44 years ($p = 0.04$), and 45-54 years ($p = 0.008$) and lower in age group 55-64 years ($p = 0.009$). Between 50 and 60 years of age there was a drop in mean circulating IGF-I bioactivity in women. Overall, before 55 years of age circulating IGF-I bioactivity in women was significantly higher, and after this age significantly lower when compared to men. Data are presented as mean ± SEM. *Significant difference between men and women within an age category. **Significant difference between men and women before or after age 55 years.

Figure 4: Relations between circulating IGF-I bioactivity measured by the IGF-I KIRA *vs.* circulating total IGF-I measured by five different IGF-I immunoassays to measure total IGF-I: **A)** IGF-I RIA, an in-house assay at University Children's Hospital, Tuebingen, Germany; **B)** IGF-I RIA-CT™, Mediagnost; **C)** IGF-I-IRMA 2800 Active®, DSL; **D)** IGF-I CIA, Nichols Advantage®; **E)** IGF-I CIA, Immulite® DPC.

Table 1: Linear regression equations: comparison of the relation between five IGF-I immunoassays.

Dependent variable(Y)	Independent variable (X)			
	A	**B**	**C**	**D**
A	***			
B	R^2 = 0.85 Y = 2.20x + 0.97	***		
C	R^2 = 0.85 Y = 1.43x – 5.38	R^2 = 0.80 Y = 1.32x – 5.58	***	
D	R^2 = 0.82 Y = 1.52x – 3.20	R^2 = 0.75 Y = 1.37x – 2.96	R^2 = 0.87 Y = 1.01x + 3.54	***
E	R^2 = 0.87 Y = 1.45x – 2.60	R^2 = 0.91 Y = 1.41x – 4.19	R^2 = 0.86 Y = 0.93x + 4.57	R^2 = 0.81 Y = 0.84 + 3.36

Regression models are based on total IGF-I levels expressed as nmol/L. In all models correlation was significant ($P < 0.001$ for all). IGF-I immunoassays: A) IGF-I RIA, an in-house assay at University Children's Hospital, Tuebingen, Germany; B) IGF-I RIA-CTTM, Mediagnost; C) IGF-I-IRMA 2800 Active®, DSL; D) IGF-I CIA, Nichols Advantage®; E) IGF-I CIA, Immulite® DPC.

Table 2: Age-related reference ranges for IGF-I bioactivity in healthy adults (N = 426)

Age (years)	**Number (N)**	**Female (N)**	**Mean (pmol/L)**	**SD (pmol/L)**	**Median (pmol/L)**	**95% CI (pmol/L)**
< 24	38	7	527	139	528	(481 - 573)
25 - 34	66	20	476	140	461	(441 - 510)
35 - 44	100	30	432	132	428	(406 - 459)
45 - 54	79	19	411	122	402	(384 - 438)
55 - 64	103	24	385	108	383	(363 - 405)
> 65	40	16	344	081	343	(318 - 370)

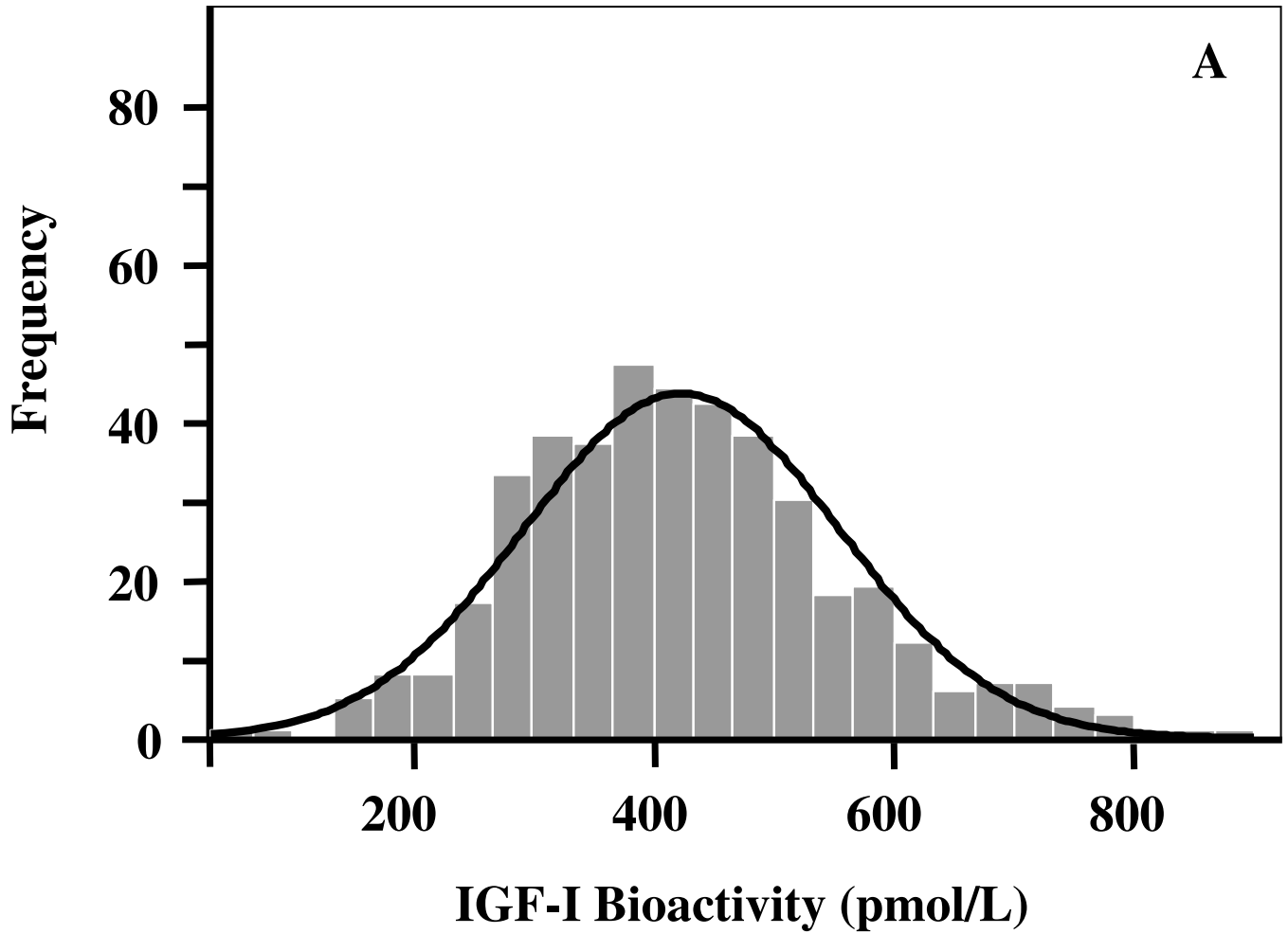
A
Frequency
80
60
40
20
0
200
400
600
800
IGF-I Bioactivity (pmol/L)

Figure 1A

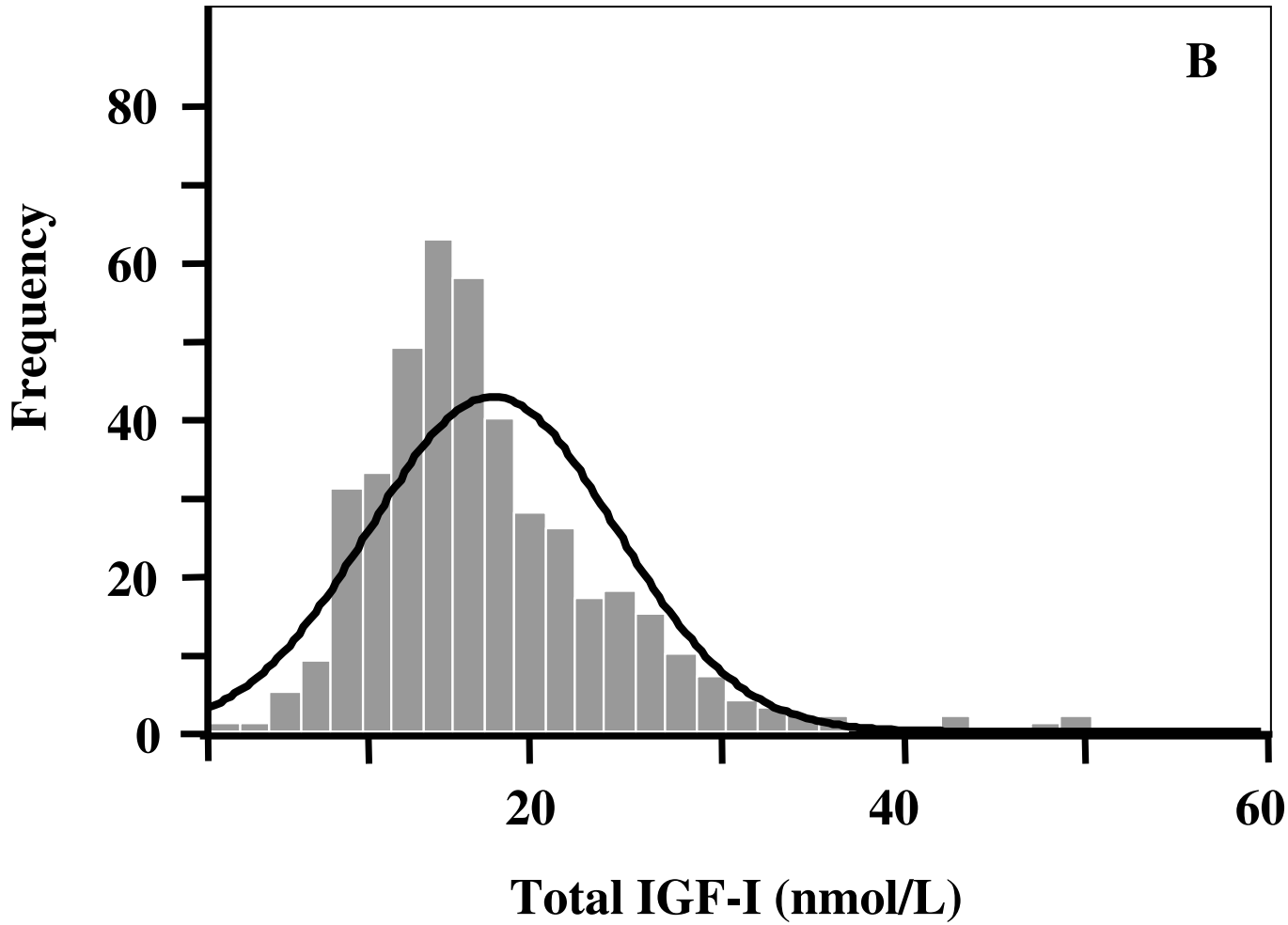

Figure 1B

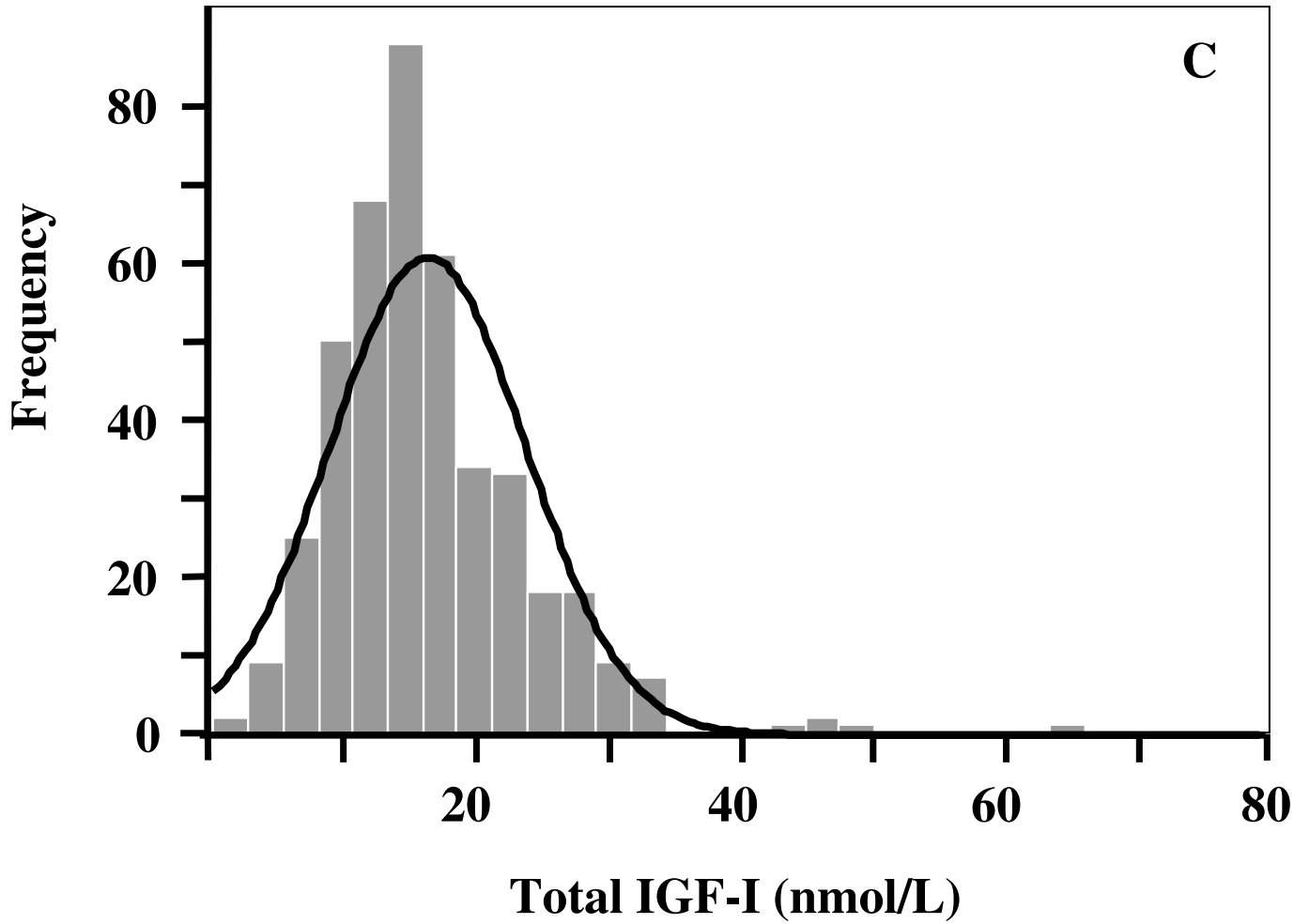

Figure 1C

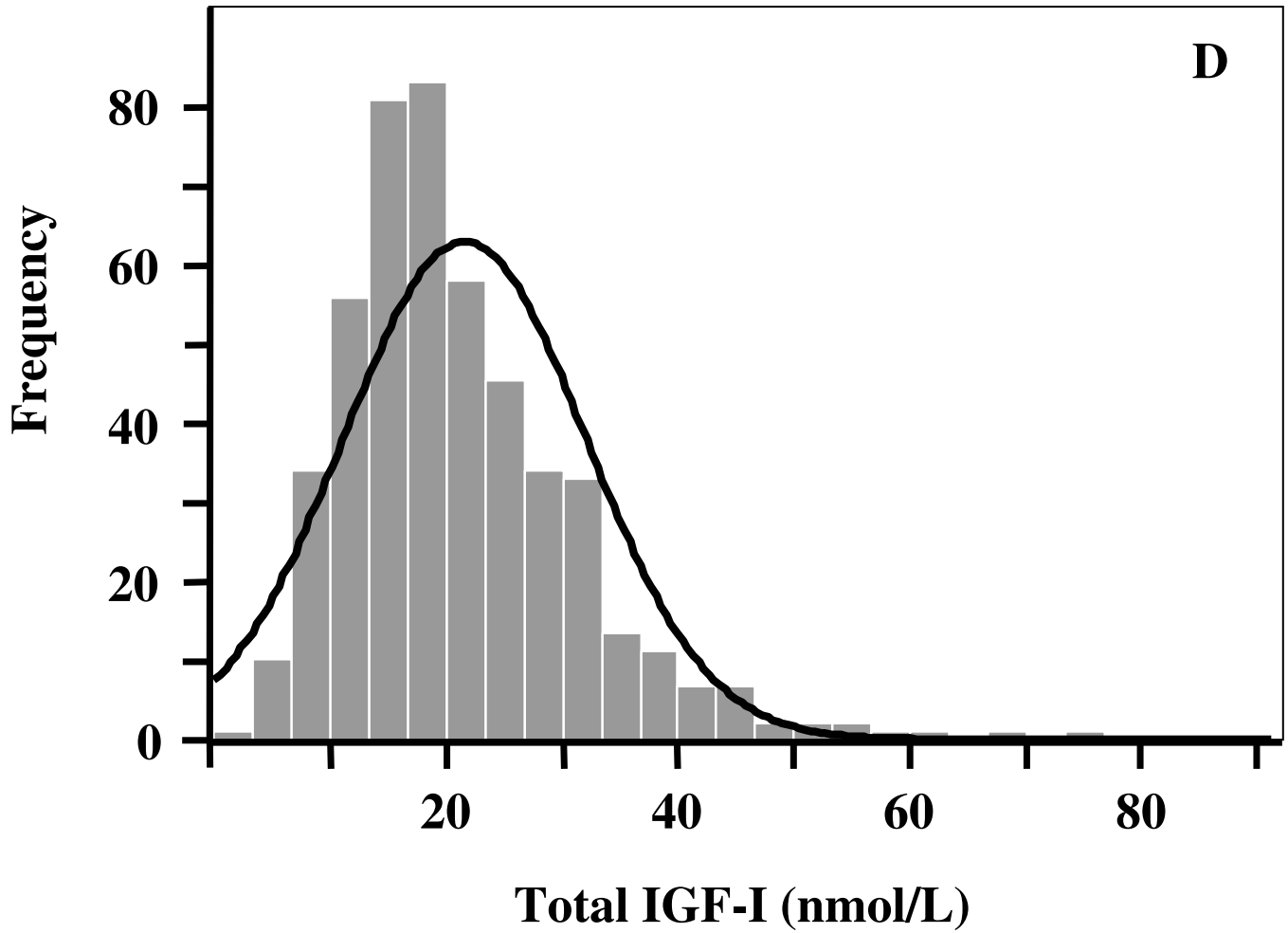

Figure 1D

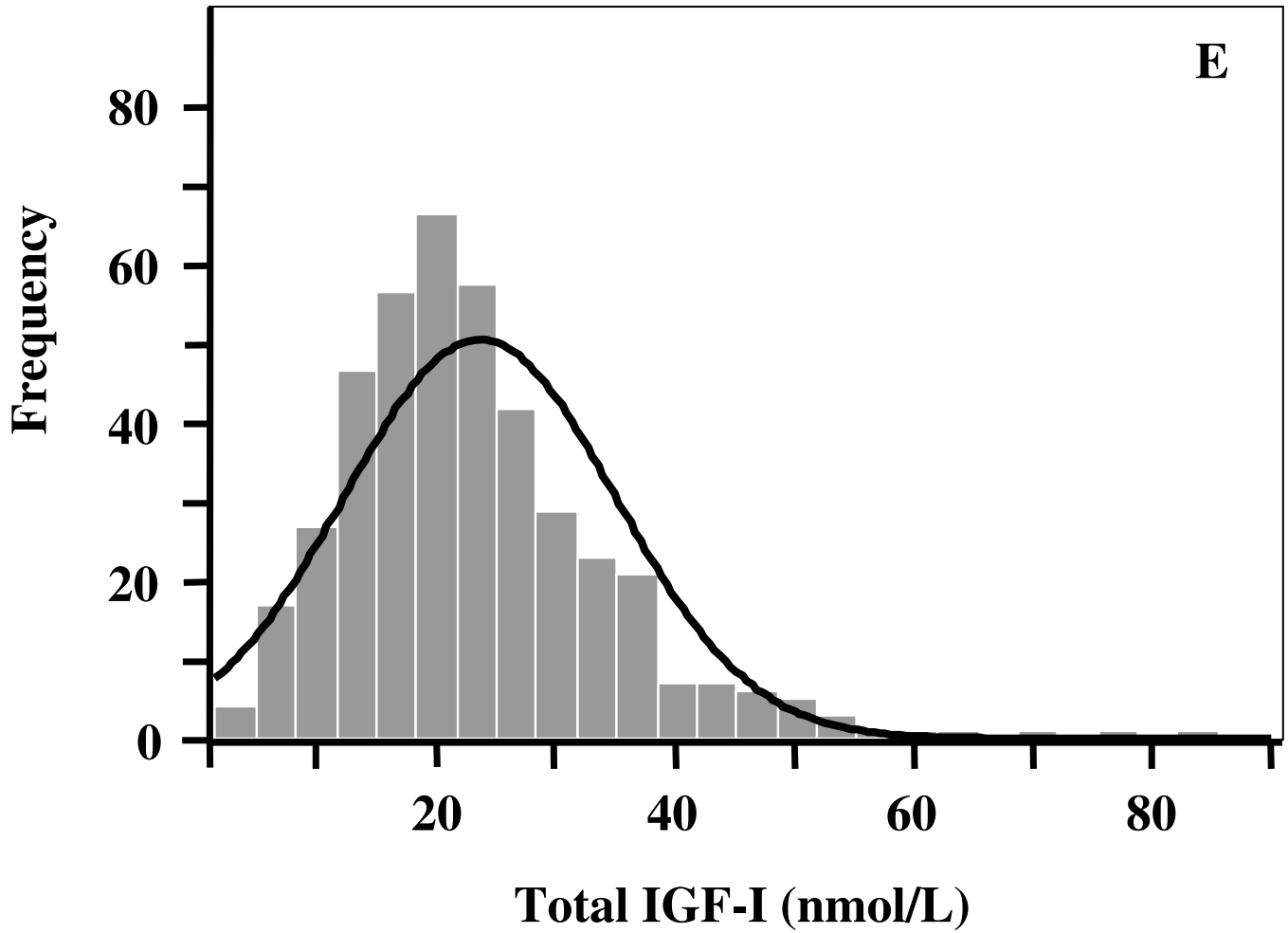

Figure 1E

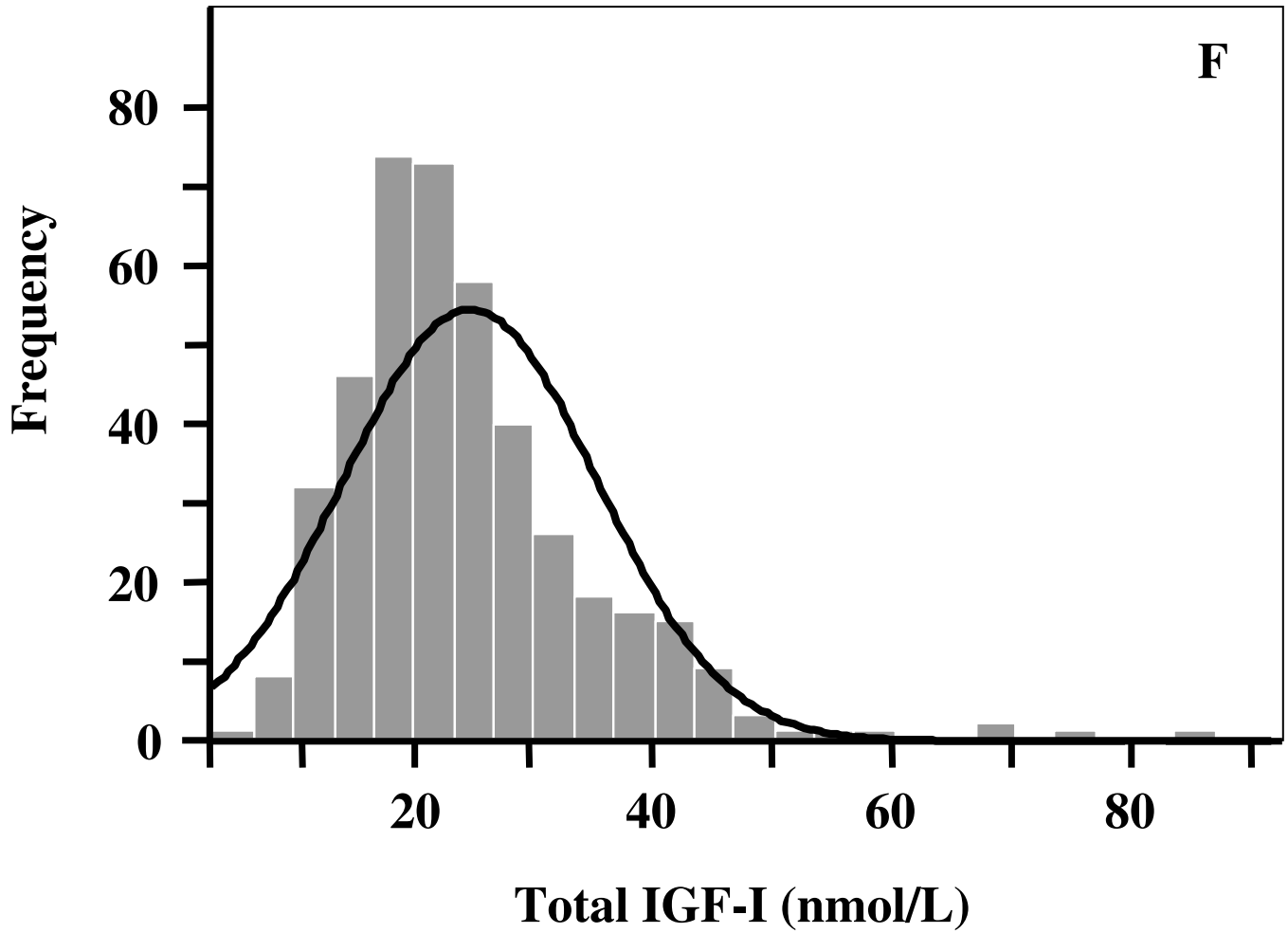

Figure 1F

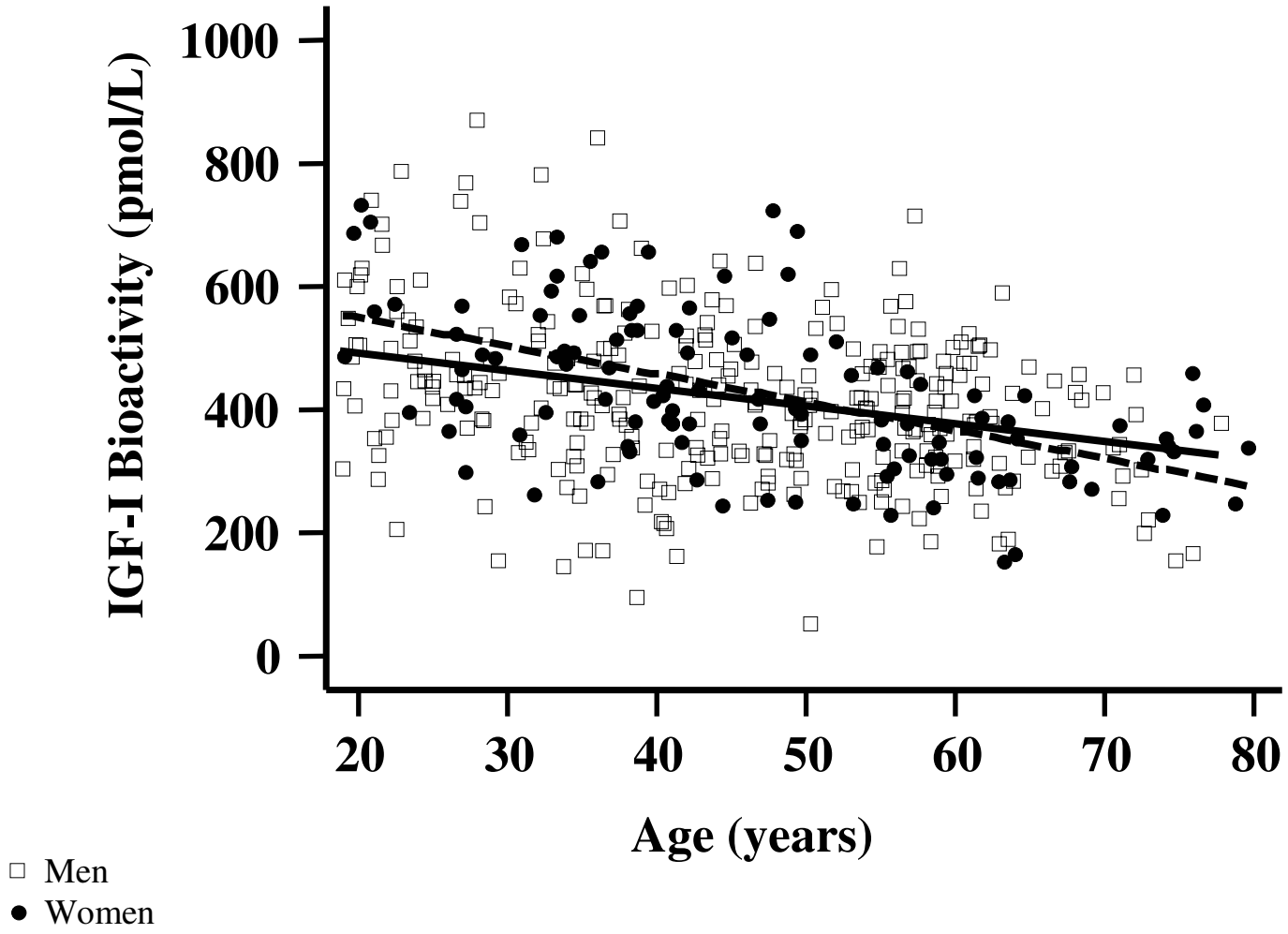
IGF-I Bioactivity (pmol/L)
1000
800
600
400
200
0
20
30
40
50
60
70
80
Age (years)
Men
Women

Figure 2

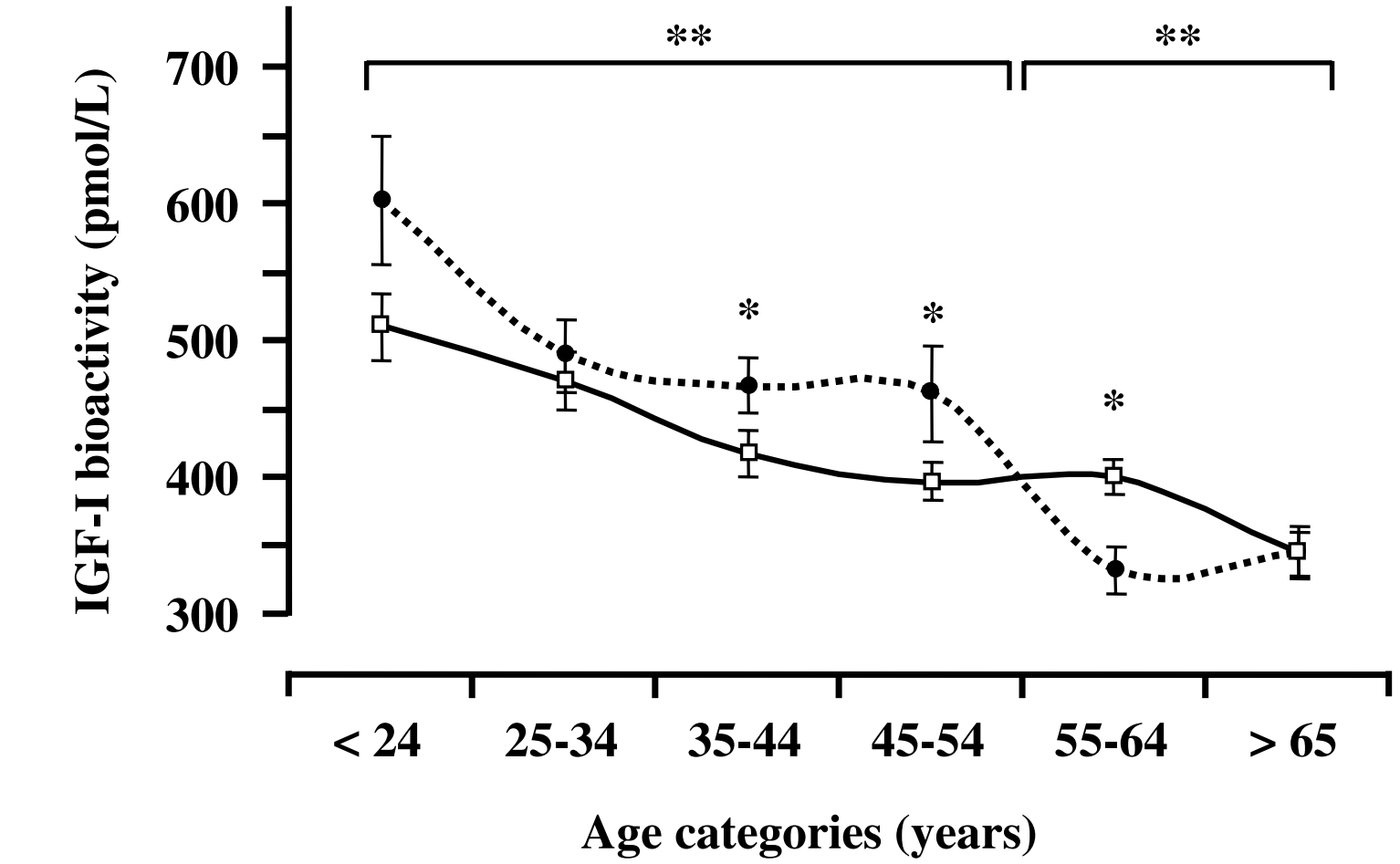
IGF-I bioactivity (pmol/L)
700
600
500
400
300
**
**
*
*
*
< 24
25-34
35-44
45-54
55-64
> 65
Age categories (years)
Men
Women

Figure 3

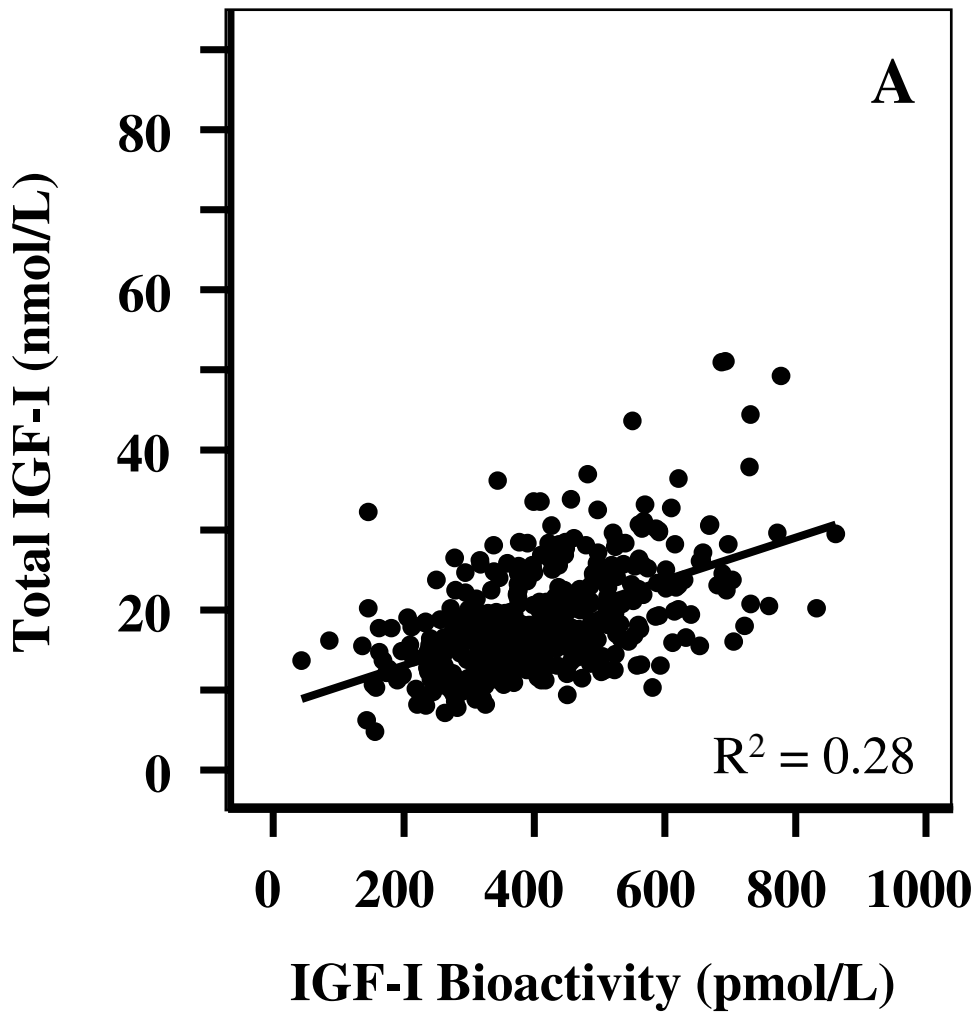
A
Total IGF-I (nmol/L)
0
20
40
60
80
0
200
400
600
800
1000
IGF-I Bioactivity (pmol/L)
R2 = 0.28

Figure 4A

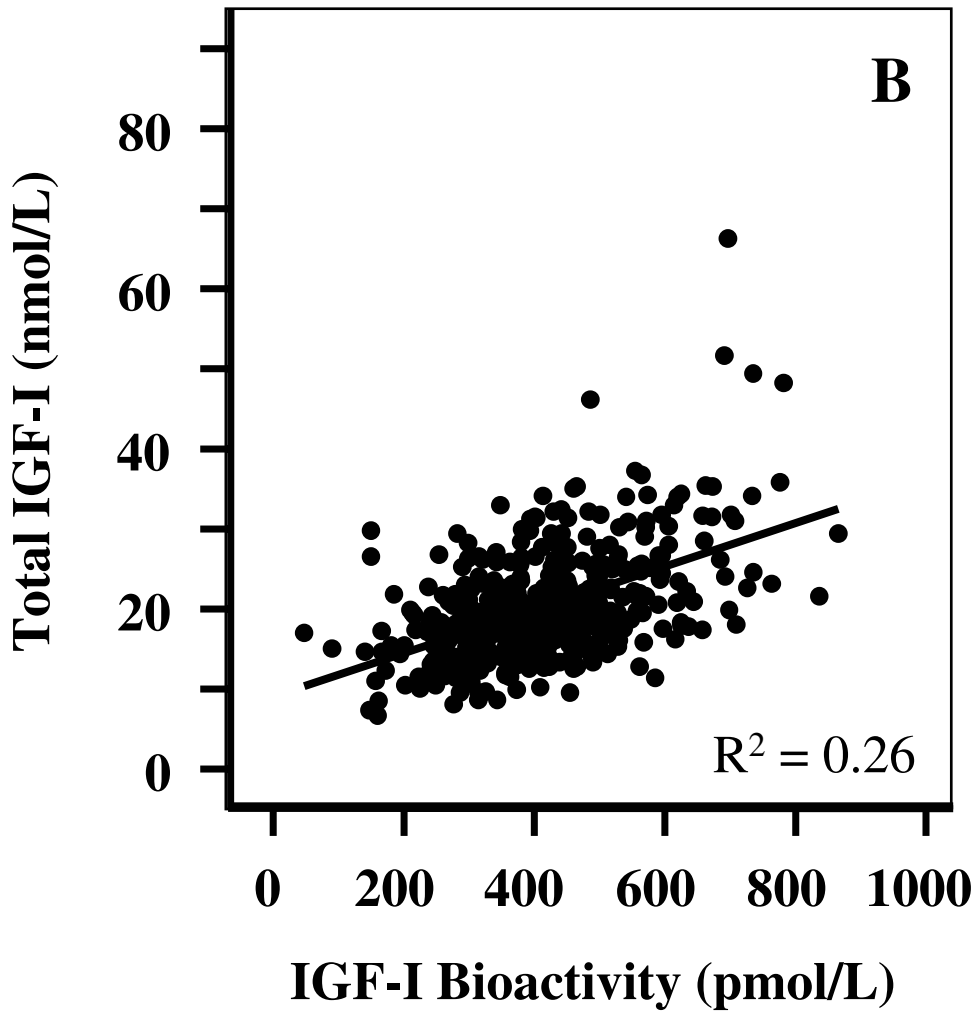
B
Total IGF-I (nmol/L)
0
20
40
60
80
0
200
400
600
800
1000
IGF-I Bioactivity (pmol/L)
R2 = 0.26

Figure 4B

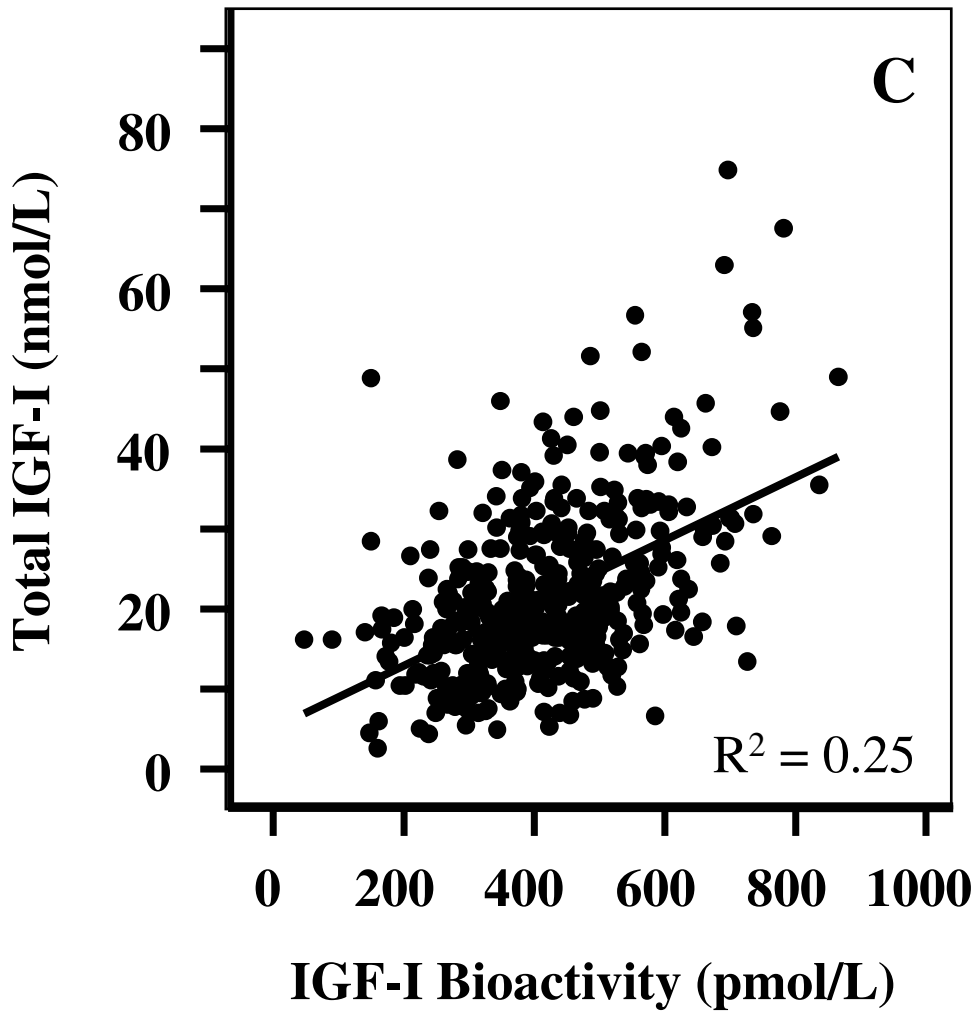
C
80
60
40
20
0
Total IGF-I (nmol/L)
0
200
400
600
800
1000
IGF-I Bioactivity (pmol/L)
R2 = 0.25

Figure 4C

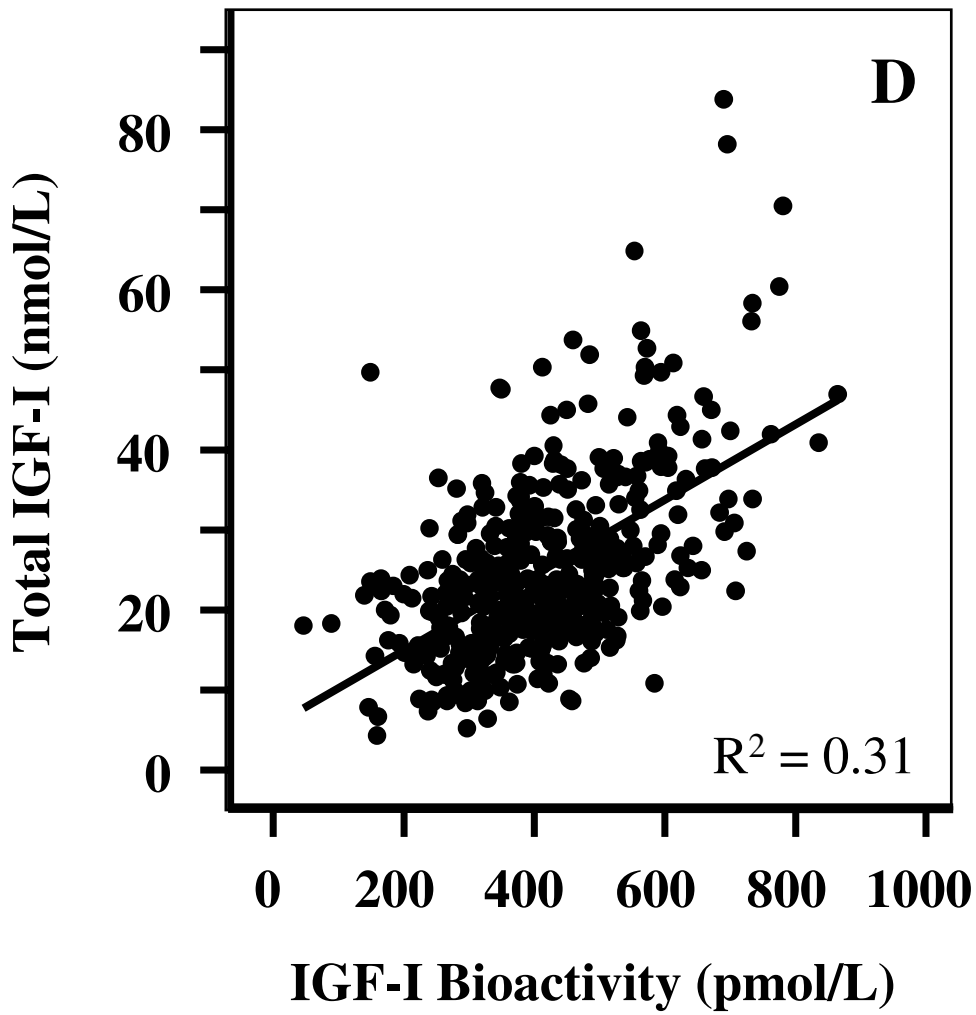
D
Total IGF-I (nmol/L)
0
20
40
60
80
0
200
400
600
800
1000
IGF-I Bioactivity (pmol/L)
R2 = 0.31

Figure 4D

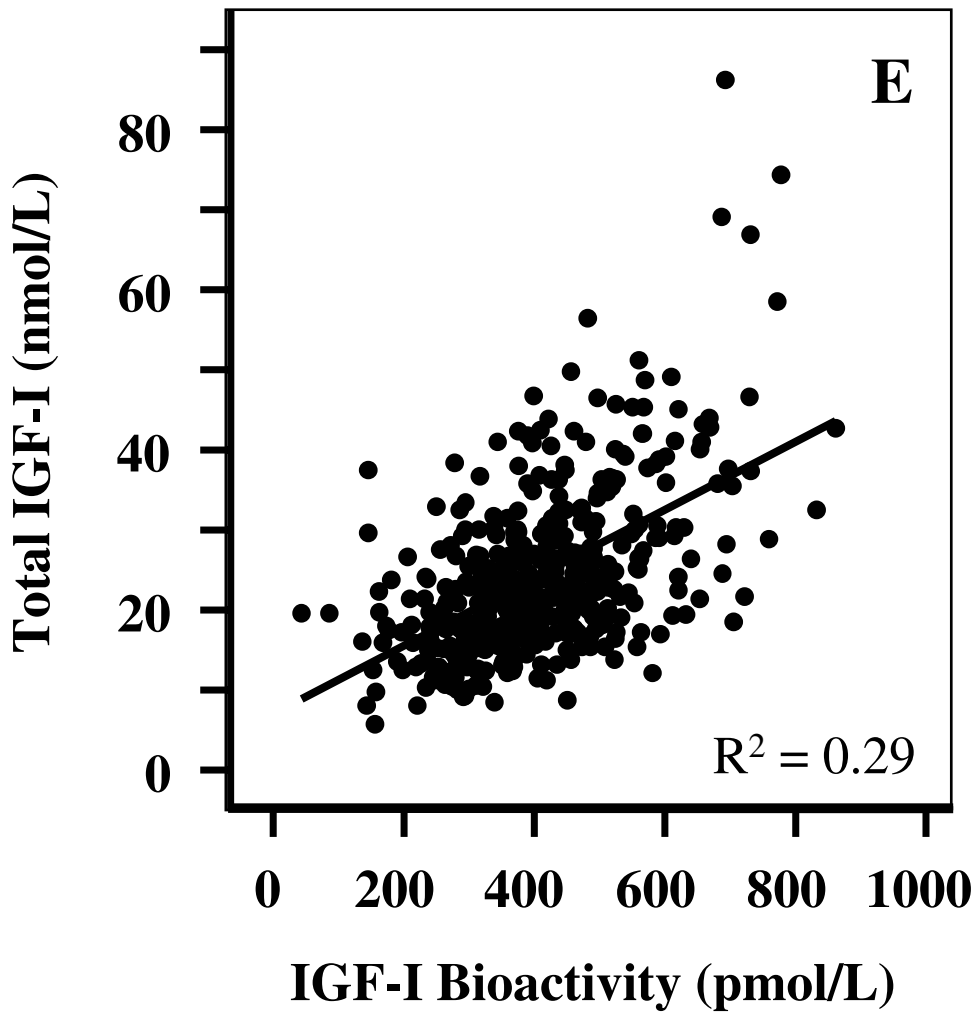
E
Total IGF-I (nmol/L)
0
20
40
60
80
0
200
400
600
800
1000
IGF-I Bioactivity (pmol/L)
R2 = 0.29

Figure 4E