

Supplemental data.

Supplemental table 1 – Centile table of total IGF-I.

Age, y	Total IGF-I Male (ng/mL)								Total IGF-I Female (ng/mL)							
	Percentile								Percentile							
	N	P5	P10	P25	P50	P75	P90	P95	P5	P10	P25	P50	P75	P90	P95	
3.5	37	72.6	84.7	109.2	144.0	188.6	239.3	275.4	84.2	97.9	125.6	165.3	217.1	277.0	320.2	
4.5	56	78.9	91.5	116.8	152.7	199.2	252.4	290.4	100.1	115.0	145.3	188.8	246.0	312.9	361.6	
5.5	48	89.0	102.4	129.4	168.0	217.9	275.3	316.7	117.3	133.7	166.9	215.0	278.7	354.3	410.2	
6.5	42	101.9	116.8	146.7	189.2	244.0	306.8	352.0	135.9	154.2	191.3	245.0	316.6	402.2	465.9	
7.5	58	116.0	132.9	166.8	214.3	275.2	344.5	393.9	155.7	176.6	219.2	281.0	363.6	462.5	536.4	
8.5	59	131.2	150.5	189.1	243.1	311.8	389.4	444.4	177.7	202.3	252.7	326.2	425.0	543.9	632.9	
9.5	64	148.8	171.2	215.8	278.2	357.1	445.8	508.3	204.4	233.8	294.3	383.3	503.8	649.9	760.1	
10.5	66	173.1	199.3	251.6	324.6	417.1	521.0	594.3	236.0	271.2	343.8	450.6	595.5	770.8	902.7	
11.5	63	209.8	240.3	301.3	387.2	497.3	622.4	711.7	268.5	310.5	396.2	519.9	683.1	874.1	1013.6	
12.5	74	258.0	293.2	363.7	463.2	591.8	739.5	845.9	299.3	348.1	445.2	579.9	748.6	935.3	1065.3	
13.5	63	310.4	350.2	429.1	539.4	680.2	840.4	955.0	324.6	379.1	484.0	622.5	786.6	958.0	1072.2	
14.5	53	358.7	402.2	486.9	602.4	745.4	903.2	1013.4	341.0	399.9	509.0	646.0	799.7	952.4	1050.3	
15.5	49	396.8	442.7	530.4	646.1	784.3	931.2	1030.6	347.0	409.3	520.4	653.4	795.7	931.1	1015.4	
16.5	63	424.3	471.6	559.9	672.8	803.0	936.6	1024.5	346.5	411.6	523.2	651.1	782.3	902.9	976.1	
17.5	43	445.7	493.6	581.1	689.9	811.4	932.3	1010.2	343.0	410.6	522.3	644.8	766.1	874.2	938.6	

Notes: The estimated percentiles are provided.

Supplemental table 2 – Centile table of total IGFBP-3.

		Total IGFBP-3 Male							Total IGFBP-3 Female						
		(ng/mL)							(ng/mL)						
		Percentile							Percentile						
Age, y	N	P5	P10	P25	P50	P75	P90	P95	P5	P10	P25	P50	P75	P90	P95
3.5	37	1917.2	2522.7	3579.8	4799.0	6052.6	7204.5	7903.2	3598.5	3985.9	4676.5	5507.1	6404.0	7267.9	7810.5
4.5	56	2211.3	2774.2	3759.3	4906.7	6100.3	7208.5	7885.7	3481.5	3922.4	4709.9	5658.0	6681.4	7665.5	8282.6
5.5	48	2491.8	3012.1	3929.2	5009.8	6147.2	7214.7	7871.9	3404.7	3894.9	4776.6	5845.5	7005.9	8126.6	8831.3
6.5	42	2732.0	3216.8	4078.8	5106.9	6202.9	7243.4	7889.3	3423.0	3937.8	4875.0	6028.6	7299.6	8543.1	9332.2
7.5	58	2922.7	3381.6	4204.3	5196.7	6267.7	7295.9	7939.6	3495.5	4014.2	4968.4	6159.0	7489.2	8807.7	9652.5
8.5	59	3076.4	3515.8	4309.4	5277.2	6334.0	7360.2	8007.8	3578.6	4096.6	5055.0	6260.9	7620.5	8980.0	9856.8
9.5	64	3193.2	3619.9	4395.5	5349.6	6401.8	7433.3	8089.1	3706.1	4221.2	5177.1	6385.0	7754.3	9131.1	10022.9
10.5	66	3256.0	3678.1	4447.4	5397.5	6450.2	7486.8	8148.2	3841.0	4354.7	5309.3	6519.1	7895.5	9285.1	10188.1
11.5	63	3263.3	3684.8	4451.2	5395.3	6438.3	7462.7	8115.1	3905.7	4418.6	5373.8	6588.1	7974.8	9380.1	10296.1
12.5	74	3215.4	3636.5	4399.0	5332.9	6358.4	7359.8	7994.8	3888.4	4394.9	5338.2	6537.9	7908.8	9299.0	10205.7
13.5	63	3133.0	3548.3	4298.6	5214.6	6217.1	7193.0	7810.4	3758.2	4255.1	5177.9	6346.7	7676.0	9017.6	9889.4
14.5	53	3023.8	3424.4	4150.3	5040.3	6018.2	6973.9	7580.1	3513.7	3997.1	4891.3	6016.8	7287.4	8560.3	9382.6
15.5	49	2869.1	3248.9	3941.7	4798.1	5747.5	6682.7	7279.5	3192.4	3653.3	4504.3	5572.1	6772.7	7970.2	8740.9
16.5	63	2695.8	3047.8	3694.1	4500.1	5402.2	6298.9	6874.9	2848.0	3275.3	4068.3	5068.9	6199.9	7332.9	8064.5
17.5	43	2520.9	2842.3	3435.5	4181.0	5022.2	5865.3	6410.4	2490.6	2883.5	3621.2	4566.1	5649.9	6750.0	7467.1

Supplemental table 3 – Centile table of intact IGFBP-3.

		Intact IGFBP-3 Male (ng/mL)							Intact IGFBP-3 Female (ng/mL)						
		Percentile							Percentile						
Age, y	N	P5	P10	P25	P50	P75	P90	P95	P5	P10	P25	P50	P75	P90	P95
3.5	37	322.2	400.0	544.1	723.2	921.2	1114.7	1237.1	344.2	445.7	624.2	832.2	1048.5	1249.0	1371.4
4.5	56	362.1	457.1	625.2	823.5	1032.0	1227.3	1347.4	372.5	486.8	683.4	906.5	1132.9	1338.7	1462.7
5.5	48	419.2	528.4	714.6	925.1	1138.5	1332.4	1449.2	412.4	536.4	746.6	981.3	1215.9	1426.8	1552.9
6.5	42	498.4	616.2	812.5	1029.2	1244.4	1437.1	1551.9	467.1	596.8	815.0	1057.2	1298.7	1515.2	1644.5
7.5	58	592.1	714.4	916.1	1136.7	1354.1	1547.7	1662.6	540.7	671.1	890.9	1136.4	1382.8	1605.0	1738.1
8.5	59	694.1	817.7	1021.1	1243.3	1462.3	1657.1	1772.7	636.2	762.8	978.9	1224.4	1474.7	1703.4	1841.7
9.5	64	789.1	914.8	1121.1	1345.9	1566.9	1762.9	1879.2	745.5	868.0	1080.5	1326.8	1582.7	1820.3	1965.6
10.5	66	874.6	1004.0	1216.2	1447.1	1673.9	1875.0	1994.1	858.7	979.5	1192.0	1442.3	1706.8	1956.1	2110.2
11.5	63	970.6	1101.4	1317.8	1555.4	1790.6	2000.4	2125.3	965.0	1087.2	1303.2	1559.5	1832.3	2091.3	2252.3
12.5	74	1071.8	1203.5	1423.7	1668.9	1914.5	2135.7	2268.3	1060.4	1185.2	1404.9	1664.7	1940.3	2201.4	2363.4
13.5	63	1154.9	1289.6	1516.8	1772.0	2029.9	2263.9	2404.8	1146.1	1272.4	1493.2	1752.0	2024.4	2280.7	2439.0
14.5	53	1226.4	1363.3	1594.1	1853.3	2114.9	2352.3	2495.1	1220.6	1347.3	1567.0	1822.3	2088.7	2337.5	2490.3
15.5	49	1280.8	1419.9	1652.9	1912.6	2172.8	2407.6	2548.2	1278.4	1405.7	1625.0	1878.0	2140.0	2383.1	2531.7
16.5	63	1314.7	1455.8	1689.5	1946.6	2201.4	2429.0	2564.5	1319.8	1449.1	1670.7	1924.0	2184.5	2424.4	2570.5
17.5	43	1341.6	1481.2	1709.8	1958.0	2201.0	2415.9	2543.0	1352.7	1485.6	1711.6	1967.9	2229.1	2468.2	2612.9

Supplemental table 4 – Centile table of PAPP-A2.

Age, y	PAPP-A2 Male (ng/mL)								PAPP-A2 Female (ng/mL)						
	Percentile								Percentile						
	N	P5	P10	P25	P50	P75	P90	P95	P5	P10	P25	P50	P75	P90	P95
3.5	37	0.164	0.197	0.259	0.345	0.458	0.604	0.724	0.172	0.203	0.259	0.334	0.438	0.583	0.720
4.5	56	0.147	0.178	0.236	0.316	0.425	0.564	0.681	0.159	0.189	0.244	0.320	0.425	0.575	0.717
5.5	48	0.132	0.160	0.214	0.289	0.390	0.521	0.629	0.148	0.177	0.231	0.306	0.409	0.557	0.696
6.5	42	0.121	0.148	0.198	0.267	0.358	0.474	0.570	0.138	0.167	0.220	0.293	0.394	0.533	0.663
7.5	58	0.114	0.139	0.186	0.250	0.332	0.434	0.516	0.130	0.159	0.212	0.282	0.376	0.502	0.614
8.5	59	0.108	0.133	0.178	0.237	0.311	0.398	0.466	0.126	0.154	0.205	0.270	0.354	0.459	0.549
9.5	64	0.105	0.129	0.173	0.227	0.292	0.366	0.421	0.121	0.149	0.197	0.256	0.329	0.416	0.487
10.5	66	0.101	0.126	0.169	0.220	0.279	0.344	0.391	0.113	0.139	0.185	0.239	0.303	0.379	0.438
11.5	63	0.094	0.119	0.163	0.214	0.270	0.331	0.374	0.099	0.124	0.167	0.217	0.277	0.347	0.402
12.5	74	0.083	0.109	0.154	0.206	0.264	0.325	0.367	0.084	0.107	0.147	0.195	0.251	0.318	0.370
13.5	63	0.072	0.099	0.145	0.199	0.260	0.325	0.371	0.073	0.094	0.130	0.175	0.228	0.290	0.340
14.5	53	0.065	0.090	0.135	0.189	0.252	0.320	0.369	0.066	0.085	0.118	0.159	0.209	0.269	0.317
15.5	49	0.060	0.080	0.119	0.168	0.229	0.297	0.348	0.061	0.078	0.109	0.148	0.196	0.256	0.306
16.5	63	0.051	0.066	0.096	0.138	0.193	0.261	0.315	0.059	0.074	0.103	0.140	0.188	0.250	0.304
17.5	43	0.039	0.049	0.071	0.104	0.152	0.221	0.283	0.058	0.072	0.097	0.132	0.178	0.242	0.300

Supplemental table 5 – Centile table of free IGF-I.

Age, y	Free IGF-I Male (ng/mL)								Free IGF-I Female (ng/mL)						
	Percentile								Percentile						
	N	P5	P10	P25	P50	P75	P90	P95	P5	P10	P25	P50	P75	P90	P95
3.5	37	0.89	1.19	1.85	2.94	4.78	7.99	11.57	0.94	1.27	1.9	2.77	3.92	5.46	6.80
4.5	56	0.93	1.22	1.85	2.91	4.74	8.06	11.95	1.00	1.35	2.02	2.94	4.22	5.96	7.52
5.5	48	0.96	1.25	1.87	2.91	4.74	8.14	12.29	1.08	1.44	2.14	3.14	4.54	6.51	8.33
6.5	42	0.98	1.27	1.89	2.93	4.75	8.10	12.15	1.17	1.54	2.29	3.35	4.88	7.09	9.17
7.5	58	1.00	1.30	1.94	2.99	4.78	7.98	11.67	1.28	1.67	2.46	3.59	5.24	7.66	9.98
8.5	59	1.01	1.33	2.00	3.08	4.86	7.85	11.10	1.41	1.83	2.65	3.86	5.61	8.23	10.77
9.5	64	1.04	1.38	2.09	3.21	4.98	7.78	10.65	1.56	2.00	2.87	4.14	6.01	8.81	11.57
10.5	66	1.10	1.47	2.23	3.39	5.15	7.83	10.45	1.71	2.18	3.1	4.42	6.39	9.35	12.30
11.5	63	1.22	1.61	2.40	3.60	5.38	8.01	10.54	1.85	2.33	3.28	4.65	6.68	9.75	12.80
12.5	74	1.37	1.78	2.61	3.82	5.61	8.25	10.77	1.93	2.43	3.39	4.77	6.79	9.82	12.82
13.5	63	1.58	1.99	2.82	4.03	5.81	8.46	11.02	1.95	2.44	3.38	4.72	6.66	9.50	12.27
14.5	53	1.79	2.21	3.02	4.21	5.97	8.66	11.33	1.87	2.35	3.26	4.54	6.34	8.94	11.39
15.5	49	1.99	2.40	3.19	4.36	6.14	8.98	11.97	1.71	2.17	3.04	4.24	5.91	8.24	10.39
16.5	63	2.17	2.56	3.33	4.49	6.34	9.49	13.12	1.51	1.94	2.76	3.88	5.40	7.46	9.31
17.5	43	2.33	2.71	3.46	4.62	6.55	10.10	14.58	1.29	1.7	2.47	3.49	4.85	6.64	8.20

Supplemental table 6 – Centile table of percentage of free IGF-I to total IGF-I.

Age, y	Percentage of f/t IGF-I Male (%)								Percentage of f/t IGF-I Female (%)							
	Percentile								Percentile							
	N	P5	P10	P25	P50	P75	P90	P95	P5	P10	P25	P50	P75	P90	P95	
3.5	37	0.71	0.91	1.34	2.02	3.08	4.71	6.3	0.49	0.7	1.09	1.63	2.34	3.29	4.09	
4.5	56	0.65	0.83	1.23	1.86	2.86	4.43	5.98	0.49	0.67	1.02	1.52	2.22	3.2	4.1	
5.5	48	0.58	0.75	1.11	1.70	2.65	4.15	5.67	0.48	0.64	0.95	1.41	2.1	3.11	4.09	
6.5	42	0.52	0.67	1.00	1.55	2.44	3.88	5.35	0.47	0.61	0.89	1.31	1.96	2.97	3.99	
7.5	58	0.45	0.59	0.90	1.40	2.23	3.58	4.97	0.45	0.58	0.83	1.22	1.82	2.76	3.74	
8.5	59	0.40	0.53	0.80	1.26	2.01	3.24	4.49	0.44	0.55	0.78	1.13	1.67	2.53	3.42	
9.5	64	0.37	0.48	0.73	1.13	1.79	2.85	3.93	0.42	0.52	0.74	1.05	1.54	2.31	3.12	
10.5	66	0.35	0.45	0.66	1.01	1.57	2.47	3.38	0.40	0.49	0.69	0.97	1.42	2.12	2.85	
11.5	63	0.33	0.42	0.60	0.90	1.38	2.13	2.9	0.37	0.46	0.64	0.89	1.29	1.93	2.6	
12.5	74	0.31	0.39	0.55	0.81	1.21	1.85	2.5	0.35	0.43	0.59	0.82	1.17	1.73	2.33	
13.5	63	0.30	0.37	0.51	0.73	1.07	1.62	2.17	0.34	0.41	0.54	0.74	1.05	1.53	2.03	
14.5	53	0.30	0.36	0.49	0.68	0.98	1.45	1.95	0.31	0.38	0.5	0.68	0.95	1.36	1.78	
15.5	49	0.31	0.36	0.48	0.65	0.93	1.39	1.88	0.29	0.35	0.47	0.63	0.88	1.25	1.61	
16.5	63	0.32	0.37	0.48	0.65	0.93	1.42	1.97	0.25	0.31	0.43	0.59	0.82	1.16	1.47	
17.5	43	0.33	0.38	0.49	0.66	0.95	1.5	2.19	0.21	0.27	0.39	0.55	0.77	1.07	1.34	

Abbreviations: f/t IGF-I, free to total IGF-I.

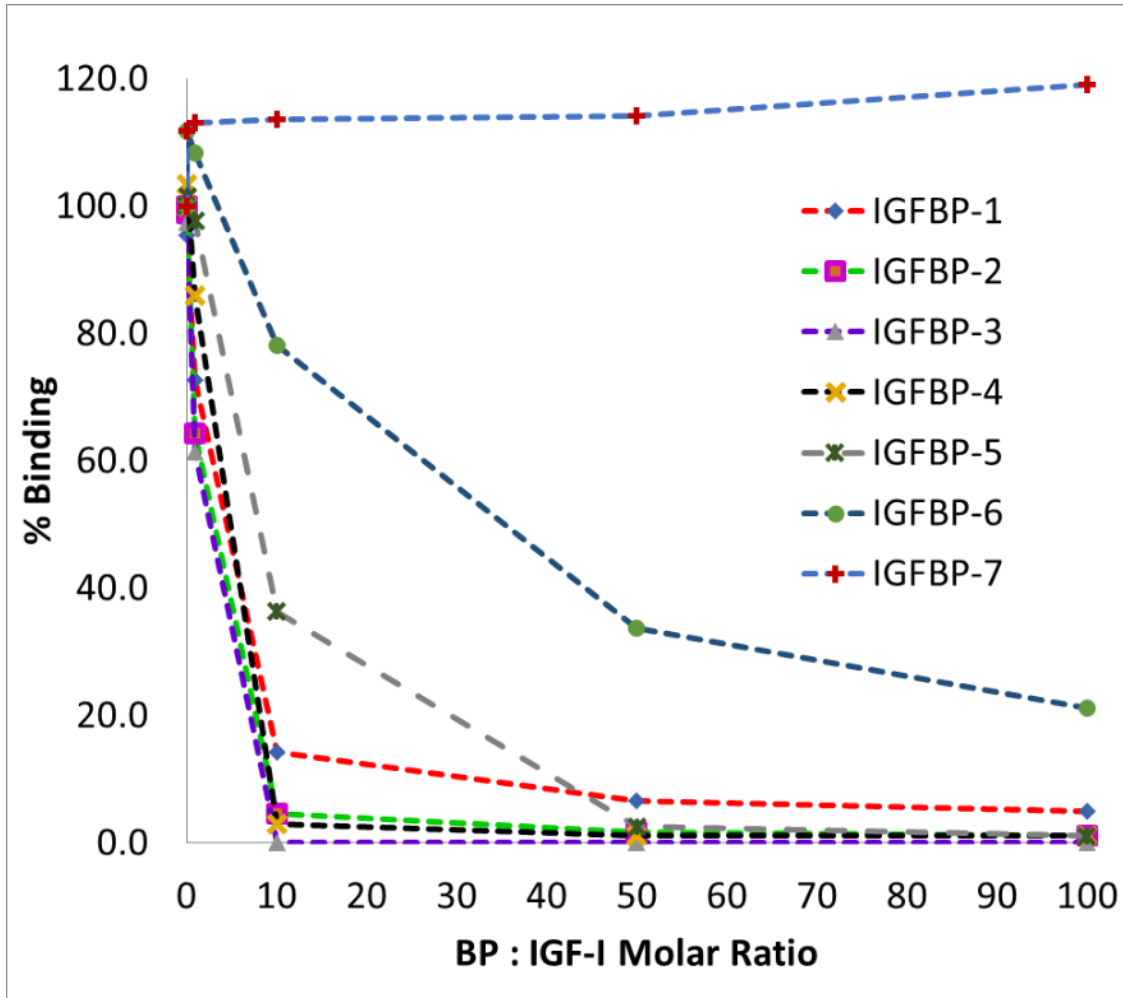
Supplemental table 7 – Comparison of mean values among breast Tanner stages.

Variable	Stage 1		Stage 2		Stage 3		Stage 4		Stage 5	
Total IGF-I[†]	273.8 ^a	(105.9)	461.2 ^b	(172.9)	800.3 ^c	(193.0)	742.7 ^c	(206.8)	626.6 ^d	(212.8)
Free IGF-I[†]	3.83 ^a	(2.67)	7.16 ^b	(15.52)	7.53 ^c	(3.93)	4.82 ^{b, d}	(2.21)	4.49 ^{a, d}	(4.07)
Percentage of free to total IGF-I[†]	1.55 ^a	(1.27)	1.49 ^a	(1.75)	0.99 ^b	(0.60)	0.67 ^c	(0.30)	0.72 ^c	(0.51)
Total IGFBP-3	6129 ^{a, b}	(1861)	6761 ^{a, c}	(1880)	7283 ^c	(1881)	5698 ^{b, d}	(1826)	5130 ^d	(1644)
Intact IGFBP-3	1088 ^a	(352.7)	1397 ^b	(385.3)	1770 ^c	(391.7)	1925 ^d	(346.1)	1940 ^d	(403.9)
PAPPA2[†]	0.33 ^a	(0.21)	0.28 ^b	(0.15)	0.20 ^c	(0.09)	0.16 ^d	(0.07)	0.15 ^d	(0.08)

[†] The natural log-transformation was used for statistical analysis. Mean (SD) was shown. Within each analyte, values with the same letter are not significantly different ($P > 0.05$) while those with different letters are significantly different ($P < 0.05$).

Supplemental Figure

Supplemental Figure S1. Inhibition of Free IGF-I measurement by IGFBPs.



Supplemental Figure S1. Inhibition of Free IGF-I measurement by IGFBPs.

Various molar ratios of IGF-I and IGFBPs were tested. All specimens contained 5 ng/mL of added rhIGF-I and were tested in the total and free IGF-I ELISAs. Inhibition of free IGF-I quantitation, which should be complete if there is no interference or release of bound IGF-I, is expressed as the percent of free IGF-I and was calculated after normalizing the unbound IGF-I measurement for each preparation with respect to the IGF-I in the absence of binding proteins.

Supplemental Figure S2. Epitope mapping of Total and Intact IGFBP-3 ELISAs.

N- Terminal	Mid Region	C-Terminal	Detection in Intact IGFBP3 ELISA	Detection in Total IGFBP3 ELISA
IGFBP5	IGFBP3	IGFBP3	+++	+++
IGFBP5	IGFBP5	IGFBP3	Not Detected	+++
IGFBP3	IGFBP5	IGFBP5	Not Detected	Not Detected
IGFBP3	IGFBP3	IGFBP5	Not Detected	Not Detected
IGFBP5	IGFBP3	IGFBP5	Not Detected	Not Detected
IGFBP3	IGFBP5	IGFBP3	Not Detected	+++

Supplemental Figure S2. Epitope mapping of Total and Intact IGFBP-3 ELISAs.

Recombinant IGFBP proteins (“chimeras”) were designed to generate 6 difference combinations of the N, Mid, or C regions of IGFBP3 and IGFBP5, as previously reported (47). Both monoclonal antibodies used in the Total IGFBP3 ELISA recognize the C-terminal of IGFBP3. The monoclonal antibodies in the Intact IGFBP3 ELISA recognize both the Mid-region and C-terminal, respectively.

1. Gaidamauskas E, Gyrupe C, Boldt HB, Schack VR, Overgaard MT, Laursen LS, Oxvig C. IGF dependent modulation of IGF binding protein (IGFBP) proteolysis by pregnancy-associated plasma protein-A (PAPP-A): Multiple PAPP-A-IGFBP interaction sites. *Biochimica et Biophysica Acta (BBA) - General Subjects*. 2013;1830(3):2701-2709.