

# Reference Intervals for Adiponectin Levels in Human Serum

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## Introduction

Adiponectin is a 30 kDa protein produced mainly by adipocytes. Plasma levels are decreased in patients with metabolic syndrome, therefore, adiponectin may serve as an early diagnostic marker to identify patients with insulin resistance.

To perform epidemiological and clinical studies to clarify the role of adiponectin in metabolic syndrome and atherogenesis validated methods and reference values in a healthy population are essential. In this study we evaluated a new ELISA system (Mediagnost, E09) for serum adiponectin and established reference intervals based on the serum levels of 520 (251 female and 269 male) healthy subjects (aged <1-65 years).

## Subjects and Methods

Adiponectin concentration was measured in blood of 520 healthy humans (Table 4). Serum was withdrawn from blood donors and children of the "Leipzig School Children Project". Blood of neonates was collected for the exclusion of metabolic diseases.

### Adiponectin ELISA

- sandwich ELISA with <2h incubation time
- use of mouse-monoclonal antibodies against adiponectin
- biotinylated detection antibody
- analytical sensitivity of 0.6 ng/ml
- intraassay coefficient of variance: 4.7%
- interassay coefficient of variance: 6.7%
- recovery of rec.adiponectin in human serum of about 100%

**Table 4: Gender dependent characterization of age (years) and BMI (kg/m<sup>2</sup>) for subjects included for the establishment of adiponectin reference intervals (µg/l).**

Female			Adiponectin (µg/ml):			
Age (Years):	n	BMI Mean ± SD	Mean ± SD	Median	Percentile 25.- 75.	Min- Max
Newborn Cord blood	19		29.8 ± 12.5	26.1	19.5-35.2	16.9-61.4
< 3.99	9	15.73 ± 0.79	14.4 ± 7.76	11.2	8.2-21.8	2.3-26.7
4.0-7.99	11	16.01 ± 1.94	8.46 ± 4.73	9.3	2.9-12.1	1.4-15.6
8.0-9.99	22	17.58 ± 3.84	7.92 ± 3.00	8.2	5.2-10.0	3.6-15.1
10.0-11.99	33	17.83 ± 1.86	7.66 ± 4.59	6.6	5.0-8.8	3.1-20.9
12.0-13.99	11	19.85 ± 2.31	8.22 ± 5.64	7.5	6.5-9.2	4.9-13.2
14.0-15.99	27	19.91 ± 1.72	8.83 ± 9.25	8.9	5.2-11.8	2.6-17.7
16.0-19.99	18	21.64 ± 2.64	9.00 ± 3.22	8.7	6.9-11.2	2.7-14.0
20.0-29.99	24	23.12 ± 5.01	7.39 ± 3.35	7.3	5.7-9.0	3.4-17.8
30.0-39.99	17	23.20 ± 2.86	9.19 ± 3.89	8.6	7.2-10.4	3.6-19.3
40.0-49.99	26	24.50 ± 4.11	9.93 ± 3.59	9.5	7.5-11.6	4.4-19.6
50.0-59.99	21	24.61 ± 3.31	11.5 ± 5.49	10.0	8.0-15.9	2.0-23.1
≥ 60.0	8	24.63 ± 1.89	15.6 ± 4.64	15.3	11.4-18.2	11.2-24.1

Male			Adiponectin (µg/ml):			
Age (Years)	n	BMI Mean ± SD	Mean ± SD	Median	Percentile 25.- 75.	Min - Max
Newborn Cord blood	10		27.8 ± 7.68	26.7	22.2-31.0	15.6-40.6
< 3.99	14	16.17 ± 1.81	16.6 ± 6.55	14.3	11.6-21.2	5.8-40.3
4.0-7.99	12	15.69 ± 1.05	11.2 ± 5.43	9.7	8.9-15.9	3.5-18.6
8.0-9.99	18	16.45 ± 1.76	8.11 ± 2.93	7.6	6.2-9.1	5.00-15.4
10.0-11.99	21	18.34 ± 2.18	8.43 ± 3.91	7.8	5.2-10.9	3.4-20.2
12.0-13.99	14	18.61 ± 2.11	7.59 ± 2.86	7.1	6.0-10.3	2.4-12.2
14.0-15.99	32	19.86 ± 2.00	7.53 ± 2.52	7.4	5.1-9.3	3.8-15.4
16.0-19.99	23	22.03 ± 2.42	7.16 ± 3.53	6.9	4.2-9.6	2.0-13.9
20.0-29.99	23	23.43 ± 2.48	5.44 ± 2.29	5.8	4.0-6.9	1.3-10.3
30.0-39.99	21	23.33 ± 2.72	5.92 ± 4.60	4.4	2.7-6.7	1.9-20.6
40.0-49.99	22	23.79 ± 2.41	6.13 ± 2.92	5.5	3.8-8.3	2.1-11.6
50.0-59.99	23	26.68 ± 2.77	7.45 ± 4.50	6.7	5.0-8.8	1.4-19.6
≥60.0	24	25.72 ± 2.12	7.48 ± 3.92	7.6	4.6-9.2	3.0-21.1

## Results

Assay characteristics like inter- and intra assay variation as well as linearity of dilution are shown in Table 1-3. Correlation analysis revealed that adiponectin concentrations of healthy subjects are strongly age-dependent whereas BMI has only a lower influence on adiponectin serum levels. Age-dependent reference values of healthy humans are presented in 13 age groups with the corresponding BMI values (Table 4).

**Table 1: Linearity of sample dilution**

Dilution:	Sample 1 (recalculated, µg/ml)	Sample 2 (recalculated, µg/ml)
1:200	12.49	11.58
1:400	11.92	11.74
1:600	10.80	11.41
1:800	11.17	11.35
1:1000	12.06	10.58
1:1200	11.64	10.96
1:1400	10.86	11.18
1:1600	10.75	10.61
<b>Mean±SD</b>	11.46 ± 0.66	11.18 ± 0.43
<b>CV (%)</b>	(5.8)	(3.8)

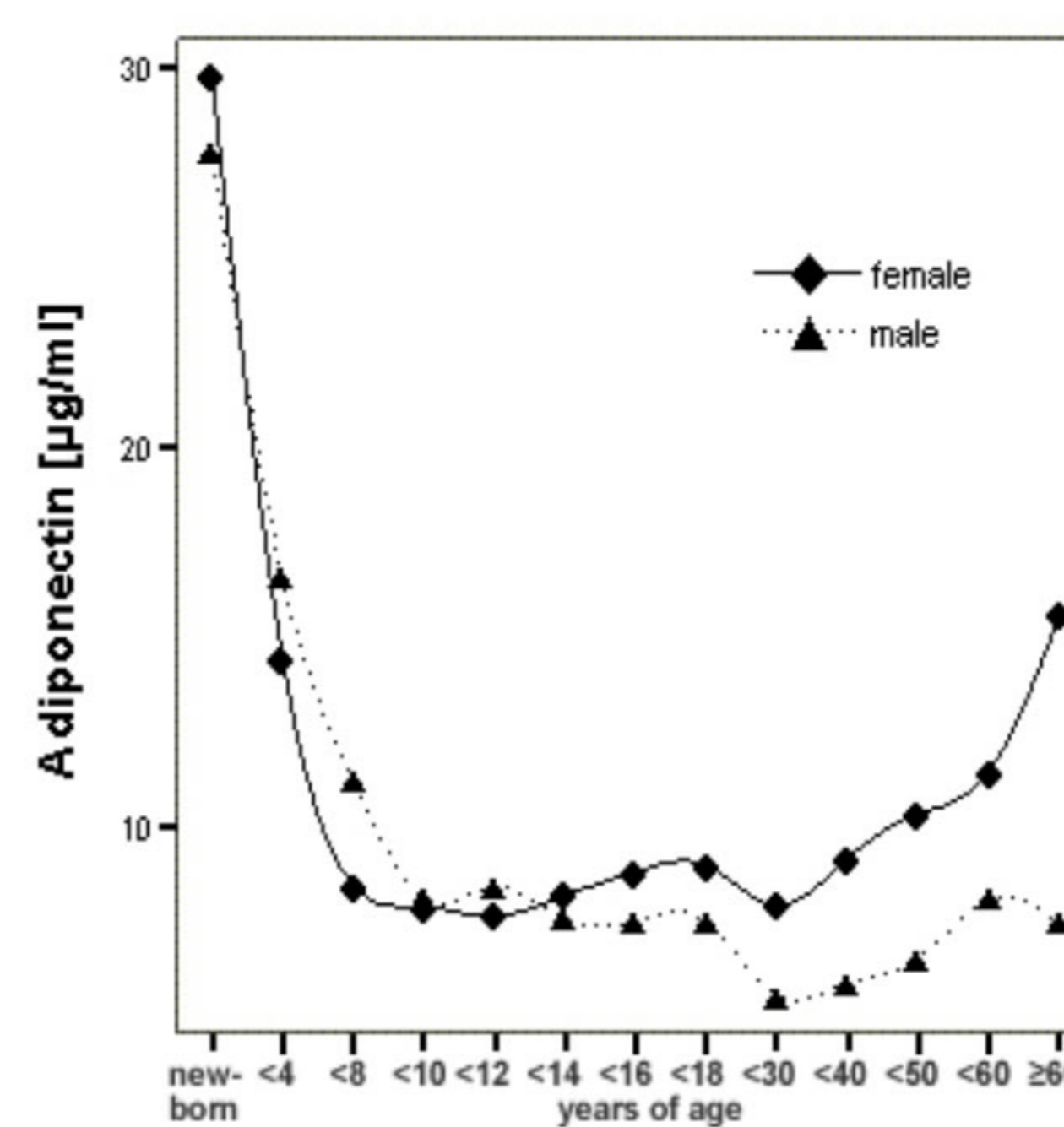
**Table 2 & 3: Inter – and Intra-Assay Variation**

Inter – Assay Variation	Number of single determinations	Mean value (µg/ml)	Standard deviation (µg/ml)	CV (%)
Sample 1	22	4.76	0.28	5.88
Sample 2	25	5.22	0.35	6.72
Sample 3	25	5.62	0.32	5.70
Sample 4	25	11.57	0.68	5.90

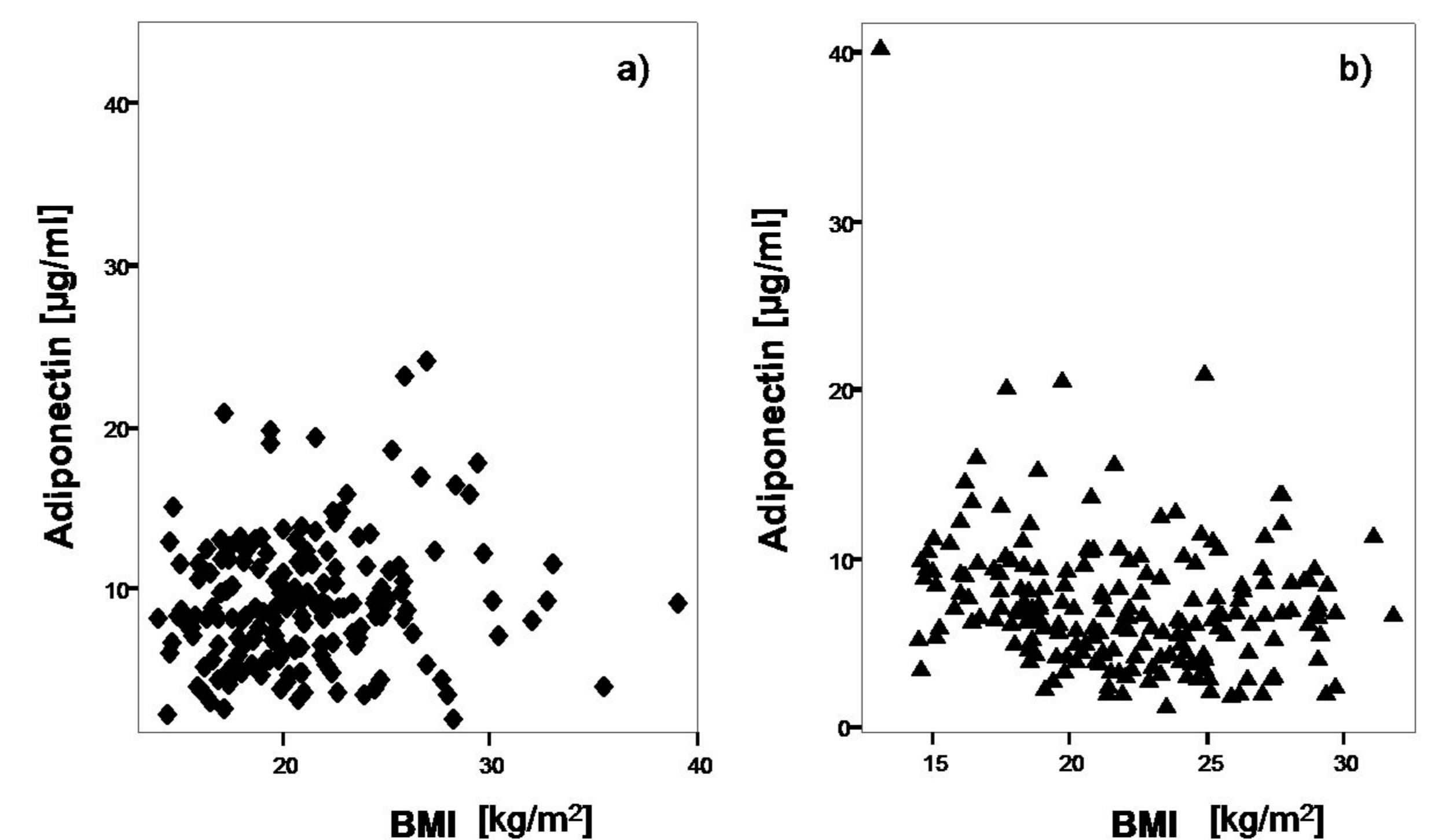
  

Intra-Assay Variation	Number of determinations	Mean value (µg/ml)	Standard deviation (µg/ml)	CV (%)
Sample 1	16	5.87	0.138	2.35
Sample 2	16	12.19	0.377	3.10
Sample 3	6	14.36	0.668	4.66

Figure 2 shows the mean adiponectin concentration of the different age groups in dependence on gender. The highest levels of this adipocytokine were found in cordblood. The median concentration was 9.0 µg/ml in female subjects (5th/95th percentile: 3.49/26.0) and 7.19 µg/ml in males (p< 0.01). Adiponectin levels and body-mass-index values of our healthy subjects are not significant correlated, neither in women nor in men (Figure 3a and b).



**Figure 2: Age dependence of adiponectin serum concentration, shown as smoothed line between mean values for each age group. Samples were pre-diluted 1:500.**



**Figure 3: Association between adiponectin levels and BMI values in men (a) and women (b). Samples were pre-diluted 1:500.**

## Summary and Conclusions

- ✓ We present for the first time age-dependent reference intervals for serum adiponectin in newborns, infants, children, adolescents and adults.
- ✓ Correlation analysis revealed that adiponectin concentrations of our healthy subjects are strongly age-dependent whereas BMI had a lower influence on adiponectin levels.
- ✓ These findings could help to establish the clinical validation of serum adiponectin levels and their predictive value for the development of metabolic syndrome and the occurrence of coronary events.