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Type 2 Diabetes in Octogenarians Is Associated with Decreased Low Molecular Weight Adiponectin

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Key Words
Adiponectin · Diabetes mellitus · Octogenarians

Abstract
Background: Adiponectin circulates in the blood in three different multimer isoforms, of which the high molecular weight form (HMW) is presumed to mediate insulin sensitivity. We examined whether adiponectin oligomer distribution is associated with aging and type 2 diabetes (T2D) in octogenarians without characteristic features of metabolic syndrome. ***Methods:*** The study included 154 octogenarians (58 men, 96 women), 24 normoglycemic middle-aged controls (11 men, 13 women; mean age 44 years), and 33 middle-aged individuals (14 men, 19 women; mean age 55 years) with T2D. Based on oral glucose tolerance test 62 octogenarians had normal, 63 impaired glucose tolerance, and 29 octogenarians had newly detected T2D. Serum adiponectin multimer isoforms were measured after overnight fast by enzyme-linked immunosorbent assays. ***Results:*** Compared to the normoglycemic middle-aged control group, male normoglycemic octogenarians revealed significantly higher total adiponectin and all adiponectin isoforms. The same was true for females with the exception of low molecular weight (LMW) adiponectin, which was not statistically higher in octogenarians. Male and female octogenarians with T2D had significantly higher levels of total, HMW, and middle molecular weight (MMW) adiponectin, but not LMW adiponectin, than middle-aged individuals with T2D. Female, but not male, octogenarians revealed significantly lower total adiponectin than normoglycemic octogenarians. Compared with normoglycemic octogenarians, male and female octogenarians with T2D were characterized by significantly lower LMW adiponectin. In male and female octogenarians, total adiponectin and all multimer isoforms were directly correlated with HDL cholesterol. LMW adiponectin in octogenarians of both sexes was inversely correlated with glucose level at 2-hour oral glucose tolerance test. ***Conclusions:*** Serum levels of total adiponectin as well as its HMW and MMW isoforms were significantly higher in octogenarians with normoglycemia or T2D than in corresponding middle-aged control groups. In male and female octogenarians without metabolic syndrome, T2D was associated with lower LMW adiponectin, while the HMW and MMW isoforms were not statistically different.

Introduction

With aging, there is an increased prevalence of insulin resistance and type 2 diabetes (T2D) resulting in a high risk for cardiovascular disease. However, humans over 80 years old are often spared age-related cardiovascular dis-

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eases, probably, in part, due to the preservation of their insulin sensitivity [1]. There is growing evidence that the adipocyte-derived hormone adiponectin plays a protective role against insulin resistance [2], atherosclerosis [3], and aging [4]. Adiponectin exerts its favorable effects on insulin sensitivity in vivo through an activation of 5′-AMP-activated protein kinase (AMPK) in skeletal muscle and liver [5]. Activated AMPK increases fatty acid oxidation and glucose uptake in myocytes and decreases hepatic glucose production by reduced expression of molecules involved in gluconeogenesis in the liver [5, 6].

Adiponectin circulates in the blood in three different isoforms: low molecular weight (LMW) trimers (about 67 kDa), middle molecular weight (MMW) hexamers (about 136 kDa), and high molecular weight (HMW) multimers (>300 kDa) [7–9]. Furthermore, globular adiponectin, a proteolytic cleavage product of adiponectin, is also present in human plasma [6]. Concentrations of HMW and MMW isoforms are significantly higher in women than in men [10], while no gender differences have been found for the LMW form [11]. Similarly to total adiponectin, HMW adiponectin has been shown to correlate directly with HDL cholesterol and inversely with triglycerides, insulin sensitivity, and inflammatory markers in T2D patients [10].

Experimental and clinical data suggest that the oligomeric complex distribution of adiponectin is essential for its anti-diabetic and anti-atherogenic activity [12, 13], and changes in the relative abundance of each oligomeric isoform in plasma may determine adiponectin activity. In subjects with metabolic syndrome, plasma levels of HMW adiponectin and the HMW to total adiponectin ratio were more effective in the prediction of insulin resistance than total adiponectin level [14]. The improvement of insulin sensitivity by thiazolidinedione treatment was rather associated with increased levels of serum HMW than total adiponectin [7]. In contrast to HMW adiponectin, the role of LMW adiponectin in the pathogenesis of T2D and insulin resistance is less defined. On the one hand, plasma LMW adiponectin was lower in obese individuals and in patients with T2D [15]. It was negatively correlated with waist circumference, body mass index (BMI) and waist-to-hip ratio (WHR) [15]. On the other hand, LMW adiponectin, like HMW and MMW adiponectin, was directly correlated with HDL cholesterol [16]. Based on this strong correlation, HDL cholesterol may account for some of the anti-diabetic and anti-atherogenic actions that have been attributed to adiponectin so far. However, there are several studies reporting biological actions of LMW adiponectin exceeding presumed effects of HDL cholesterol. Thus, LMW adiponectin specifically reduced monocytic IL-6 secretion in vitro [15, 17]. This in turn might attenuate obesity-induced systemic inflammation in vivo.

Apart from insulin sensitization of peripheral tissues, adiponectin has been shown to exert significant effects on energy homeostasis via central mechanisms. Treatment of mice with intracerebroventricular injections of adiponectin resulted in decreased body weight and fat content mediated by hypothalamic AdipoR1 receptors [18, 19]. The total adiponectin concentration in human cerebrospinal fluid approximates 0.1% of the plasma concentration [20, 21], but LMW adiponectin is the predominant adiponectin isoform in cerebrospinal fluid [20, 21]. Thus, LMW adiponectin through its central action may exert a much higher biological significance than is currently acknowledged.

The present study aimed to characterize the metabolic state of a group of octogenarians by an oral glucose tolerance test (OGTT) and to elucidate the interrelated effects of aging, sex, and insulin resistance on plasma adiponectin isoform distribution.

Research Design and Methods

Study Population

One hundred and fifty-four octogenarians (58 men, age 82.9 ± 3.8 years; 96 women, age 83.2 ± 4.3 years) without a personal or family history of T2D among first-degree relatives were enrolled at the Spreewaldklinik Rehabilitation Hospital, Burg (Spreewald), Germany. The study was performed in accordance with the Declaration of Helsinki and approved by the local Ethics Committee. Written informed consent was obtained from every subject. Patients with known T2D, plasma LDL cholesterol ≥4.5 mM, triglycerides ≥2.6 mM, heart failure and severe chronic diseases, as well as postoperative status were excluded from the study.

For comparison, 24 normoglycemic middle-aged control individuals (aged 44 ± 7 years, 11 men, 13 women) and 33 middle-aged individuals with T2D (aged 55 ± 8 years, 14 men, 19 women) were recruited.

All individuals underwent a standardized clinical examination, and current medication was recorded. Blood samples were taken after 12-hour overnight fast for routine laboratory analyses. Subsequently, a standard 75 g OGTT was performed. During OGTT, plasma levels of glucose, insulin, C-peptide, proinsulin and free fatty acids were determined at 0, 30, 60, 90, and 120 min. Serum samples for adiponectin and hormone measurements were prepared immediately, shock frozen in liquid nitrogen and stored at −80°C until analysis. Impaired glucose tolerance (IGT) and T2D were diagnosed according to the ADA and World Health Organization criteria [22]. Normal glucose tolerance (NGT) was diagnosed in 62 (24 men, 38 women), IGT in 63 (24 men, 39 women) octogenarians. T2D was newly diagnosed in 29 (10 men, 19

women) octogenarians, which represented 18.8% of the investigated octogenarian population.

According to Matthews' [23] 'homeostasis model assessment of insulin resistance' (HOMA-IR) as an index for insulin resistance was calculated by the formula: (fasting insulin [μU/ml] × fasting glucose [mM])/22.5.

Laboratory Analyses

Plasma triglycerides, total cholesterol, HDL, and LDL cholesterol were determined by standard methods on a modular analyzer (Roche, Indianapolis, Ind., USA), free fatty acids on a Cobas Mira analyzer (Global Medical Instrumentation, Inc., Ramsey, Minn., USA), and plasma glucose on a DX80 analyzer (Beckman-Coulter, Fullerton, Calif., USA). HbA_{1C} was measured by fully automated HPLC system (Bio-Rad Laboratories, Richmond, Calif., USA). Plasma levels of insulin and C-peptide were determined by enzyme-linked immunosorbent assays (ELISA) from BioSource (BioSource Europe SA, Belgium), and proinsulin by an ELISA from DRG Diagnostics (Marburg, Germany).

Assay for Adiponectin Multimer Complexes

The measurement of adiponectin multimer complexes was performed using the Adiponectin (Multimeric) ELISA from ALPCO Diagnostics (Salem, N.H., USA) for quantitative and selective determinations of HMW, MMW, and total adiponectin in serum. This assay is able to directly quantify total adiponectin, HMW + MMW and HMW. Concentrations of LMW and MMW were obtained by subtracting HMW + MMW from total adiponectin and HMW from HMW + MMW, respectively. The ELISA, which uses two monoclonal antibodies, had a linear range of 0.075–4.8 ng/ml. Intra-assay coefficients of variations for total, HMW + MMW, and HMW adiponectin were reported to be 5.3, 4.1 and 3.3%, respectively. The interassay coefficients of variations were 5.0, 6.0 and 5.7% for total, HMW + MMW, and HMW adiponectin, respectively. Each of the three forms of adiponectin multimer levels closely correlated with the total adiponectin levels in healthy subjects [11].

Statistics

Differences of basal anthropometric and clinical data in men and women among multiple groups (octogenarians with NGT, with IGT or type 2 diabetes mellitus, and middle-aged (younger) individuals with NGT or type 2 diabetes mellitus) were analyzed by univariate analysis of variance (ANOVA) and post-hoc Bonferroni tests.

To avoid significant pharmacological effects on study results, specific drug effects were excluded by a separate analysis of variance preceding subsequent statistical evaluation.

Comparisons of serum levels of separate adiponectin isoforms among sex-stratified groups of octogenarians and controls were realized by a univariate model of variance with age and BMI as covariates (ANCOVA) and post-hoc testing with p values corrected for multiple testing (Bonferroni procedure). Complex interaction of adiponectin isoforms and all groups of investigation was analyzed using a model of multivariate analysis of variance with values adjusted for sex, age and BMI. Group differences were subsequently tested by univariate analysis of variance with p values corrected for multiple testing (Bonferroni procedure).

Partial correlation tests were applied to evaluate the strength of association between total adiponectin and adiponectin isoforms with clinical data. The correlation has been controlled for BMI. Partial r and two-tailed significance have been indicated.

Data are given as mean with 95% confidence interval, unless otherwise stated. A value of $p < 0.05$ was considered statistically significant. All statistical analyses were performed with the SPSS statistical package (v16.0 for Windows; SPSS, Chicago, Ill., USA).

Results

Basic Characteristics of the Study Population

Anthropometric data and baseline metabolic parameters of octogenarians and control subjects are given in tables 1 (men) and 2 (women). Middle-aged individuals with T2D of both sexes displayed characteristic diabetes-associated changes in comparison to middle-aged normoglycemic controls, like higher values for BMI, systolic blood pressure, HbA_{1c}, HOMA-IR and plasma triglycerides (tables 1, 2). In contrast, these parameters were not significantly different between octogenarians with T2D and octogenarians with NGT of both sexes. These data led to the suggestion that although octogenarians with T2D fulfill the classification criteria for diabetes mellitus, based on fasting blood glucose and 2-hour postchallenge glucose level, they represent a specific pathogenetic entity. In order to proof this assumption, the time course of insulin, C-peptide, and proinsulin during OGTT have been analyzed. In octogenarian men with T2D, an attenuated increase in insulin at 30 min OGTT indicated a delayed response to glucose challenge (fig. 1). In contrast to middle-aged individuals with T2D, in whom maximum insulin level was observed 90 min after glucose load, the insulin level in octogenarians with T2D increased until the end of the observation period (fig. 1). In accordance, comparable kinetics could be demonstrated for C-peptide curves (online suppl. table A, www.karger.com/doi/10.1159/000316575). In middle-aged individuals with T2D, the level of proinsulin reached a maximum also at 90 min OGTT, which remained unchanged up to 120 min (online suppl. table B). The absolute values of proinsulin at 90 min in octogenarians with T2D tended to be lower, but increased further until 120 min (online suppl. table B). Statistically significant differences of proinsulin/insulin ratios during OGTT between octogenarians and middle-aged controls with T2D could not be established (online suppl. table C).

Taken together, these observations underline the specificity of the investigated octogenarian population. In particular, octogenarians with T2D do not reveal obvious characteristics of the metabolic syndrome or beta-cell insufficiency. It is suggested that a combination of reduced

Table 1. Basal anthropometric and clinical data of male octogenarians with NGT (NGT-Oc), IGT (IGT-Oc), and type 2 diabetes mellitus (T2D-Oc), and of middle-aged (younger) male normoglycemic controls (NGT-Y) and individuals with type 2 diabetes (T2D-Y)

		NGT-Oc (n = 24)	IGT-Oc (n = 24)	T2D-Oc (n = 10)	NGT-Y (n = 11)	T2D-Y (n = 14)	p
Age, years	mean	83.0	83.0	82.7	43.3	55.5	<0.001[1, 2]
	95% CI	82.1–84.0	82.2–83.8	81.2–84.3	37.2–49.4	50.9–60.1	
BMI	mean	26.4	24.0	26.9	25.2	29.4	<0.001[3]
	95% CI	25.1–27.7	22.9–25.1	24.1–29.8	24.5–25.9	27.7–31.0	
WHR	mean	0.95	0.94	0.98	0.91	0.98	0.011[4]
	95% CI	0.93–0.98	0.92–0.96	0.93–1.03	0.87–0.94	0.96–1.01	
RR syst., mm Hg	mean	135	133	126	127	145	0.007[5]
	95% CI	129–141	128–138	116–137	116–139	136–155	
RR diast., mm Hg	mean	81	78	72	73	84	0.014
	95% CI	77–85	73–83	63–80	67–80	77–90	
HbA_{1c}, %	mean	5.5	5.4	5.9	5.3	6.2	<0.001[3]
	95% CI	5.4–5.7	5.2–5.6	5.5–6.2	5.0–5.6	5.9–6.5	
HOMA-IR	mean	2.5	3.3	3.1	2.0	5.3	<0.001[2]
	95% CI	2.0–3.0	2.7–3.9	2.1–4.2	1.4–2.6	3.5–7.1	
TG, mM	mean	0.96	1.17	1.19	1.41	2.21	<0.001[6]
	95% CI	0.86–1.06	1.01–1.32	0.78–1.60	1.08–1.75	1.19–3.22	
Total chol., mM	mean	4.30	4.29	4.47	5.32	5.03	0.011[7]
	95% CI	3.86–4.74	3.74–4.83	3.88–5.06	4.60–6.03	4.45–5.61	
HDL-C, mM	mean	1.31	1.30	1.28	1.57	1.21	0.074
	95% CI	1.15–1.48	1.01–1.58	1.09–1.48	1.36–1.78	1.09–1.34	
LDL-C, mM	mean	2.61	2.50	2.68	3.50	3.13	0.015[7]
	95% CI	2.23–2.98	2.13–2.87	2.07–3.29	2.89–4.10	2.62–3.63	
FFA, mM	mean	0.43	0.51	0.66	0.56	0.53	0.173
	95% CI	0.32–0.55	0.42–0.60	0.44–0.88	0.29–0.83	0.43–0.63	

TG = Plasma triglycerides; FFA = free fatty acids. Group differences tested by univariate analysis of variance; Bonferroni test $p \leq 0.05$: [1] NGT-Y vs. all; [2] T2D-Y vs. all; [3] T2D-Y vs. NGT-Oc, IGT-Oc, NGT-Y; [4] T2D-Y vs. NGT-Y; [5] T2D-Y vs. NGT-Y, T2D-Oc; [6] T2D-Y vs. NGT-Oc, IGT-Oc, T2D-Oc; [7] NGT-Y vs. IGT-Oc.

peripheral glucose consumption and moderately altered beta-cell function may result in a diabetic state, which is different from the situation in younger diabetic individuals with obesity and clustered metabolic deteriorations.

Total Serum Adiponectin and Adiponectin Multimer Isoforms

Effect of Age

In comparison to the middle-aged normoglycemic male control group, serum concentrations of total adiponectin and all adiponectin isoforms were higher in normoglycemic octogenarian men (table 3). In octogenarian men, the HMW/total adiponectin ratio was higher (41.7 vs. 36.7%), the LMW/total adiponectin ratio lower (36.0 vs. 39.1%) than in normoglycemic middle-aged men. Male octogenarians with T2D revealed significantly higher serum levels of total adiponectin, HMW adiponectin, and MMW adiponectin than middle-aged men with T2D. LMW adiponectin was not different between these two groups.

Similarly, serum concentrations of all adiponectin isoforms were higher in normoglycemic octogenarian women, with the exception of LMW adiponectin that did not reach significance (table 3). Furthermore, the HMW/total adiponectin ratio was higher (47.5 vs. 43.1%), the LMW/total adiponectin ratio was significantly lower in normoglycemic octogenarian women (27.8 vs. 36.7%, p = 0.014), when compared with normoglycemic middle-aged women. Like in men, female octogenarians with T2D had significantly higher serum levels of total adiponectin, HMW adiponectin, and MMW adiponectin than middle-aged women with T2D (table 3).

Table 2. Basal anthropometric and clinical data of female NGT-Oc, IGT-Oc, T2D-Oc, NGT-Y and T2D-Y

		NGT-Oc (n = 38)	IGT-Oc (n = 39)	T2D-Oc (n = 19)	NGT-Y (n = 13)	T2D-Y (n = 19)	p
Age, years	mean	83.3	83.3	82.4	44.3	54.2	<0.001[1, 2]
	95% CI	81.9–84.7	82.2–84.3	80.8–84.0	41.7–46.9	50.2–58.2	
BMI	mean	26.5	25.4	28.3	24.1	32.0	<0.001[3]
	95% CI	25.0–27.9	23.8–26.9	25.5–31.0	22.9–25.3	28.3–35.7	
WHR	mean	0.91	0.91	0.90	0.85	0.92	0.117
	95% CI	0.88–0.93	0.89–0.94	0.86–0.93	0.78–0.91	0.89–0.95	
RR syst., mm Hg	mean	136	140	134	120	140	0.007[1]
	95% CI	132–141	134–146	126–142	109–131	131–149	
RR diast., mm Hg	mean	77	76	81	71	86	0.005[4]
	95% CI	73–80	72–80	76–86	62–80	80–92	
HbA_{1c}, %	mean	5.6	5.4	6.0	5.3	6.2	<0.001[3, 5]
	95% CI	5.3–5.9	5.3–5.5	5.7–6.3	5.1–5.4	6.0–6.5	
HOMA-IR	mean	3.0	3.0	3.8	2.2	6.2	<0.001[2]
	95% CI	2.4–3.6	2.5–3.4	3.0–4.6	1.6–2.8	4.7–7.7	
TG, mM	mean	1.22	1.39	1.55	1.01	1.84	0.003[4]
	95% CI	1.12–1.33	1.21–1.58	1.23–1.88	0.74–1.29	1.29–2.39	
Total chol., mM	mean	5.29	5.35	5.02	5.12	5.24	0.847
	95% CI	4.99–5.58	5.00–5.71	4.57–5.47	4.64–5.60	4.84–5.64	
HDL-C, mM	mean	1.49	1.48	1.31	2.15	1.44	<0.001[1]
	95% CI	1.37–1.61	1.35–1.62	1.18–1.44	1.91–2.40	1.24–1.63	
LDL-C, mM	mean	3.27	3.28	3.02	2.87	3.20	0.599
	95% CI	3.00–3.54	2.98–3.59	2.64–3.40	2.45–3.29	2.82–3.57	
FFA, mM	mean	0.61	0.67	0.67	0.54	0.79	0.044
	95% CI	0.53–0.69	0.59–0.76	0.55–0.79	0.43–0.66	0.67–0.91	

Group differences tested by univariate analysis of variance; Bonferroni test $p \leq 0.05$: [1] NGT-Y vs. all; [2] T2D-Y vs. all; [3] T2D-Y vs. NGT-Oc, IGT-Oc, NGT-Y; [4] T2D-Y vs. NGT-Y; [5] IGT-Oc vs. T2D-Oc.

Effect of Sex

Women of the octogenarian NGT group had significantly higher total, HMW, and MMW adiponectin than men in the corresponding octogenarian NGT group. Serum LMW adiponectin of both groups was not statistically different (table 3). The same tendencies could also be detected in the group of middle-aged individuals with normoglycemia and in the octogenarian IGT group showing significantly higher levels in women of total, HMW, and LMW adiponectin, but no significant differences for MMW multimers.

Significantly higher serum concentrations of total and HMW adiponectin were found in middle-aged women with T2D in comparison to men of the same group. In contrast, no differences could be established between male and female octogenarians with T2D.

Effect of T2D

Middle-aged men with T2D revealed a tendency of lower total, HMW, and MMW adiponectin, which did not reach statistical significance (table 3). Unlike men, middle-aged women with T2D were characterized by a tendency for lower total and LMW adiponectin. This tendency was enhanced in female octogenarians with T2D, resulting in significantly lower LMW adiponectin. In addition, female octogenarians with T2D displayed also a tendency of lower HMW adiponectin. Taken together, these changes resulted in significantly lower total adiponectin in female octogenarians with T2D (table 3).

In accordance, significantly lower values for LMW adiponectin could also be observed in male octogenarians with T2D in comparison with the normoglycemic octogenarian group (table 3). In contrast to female octogenarians, these changes were not accompanied by lower HMW

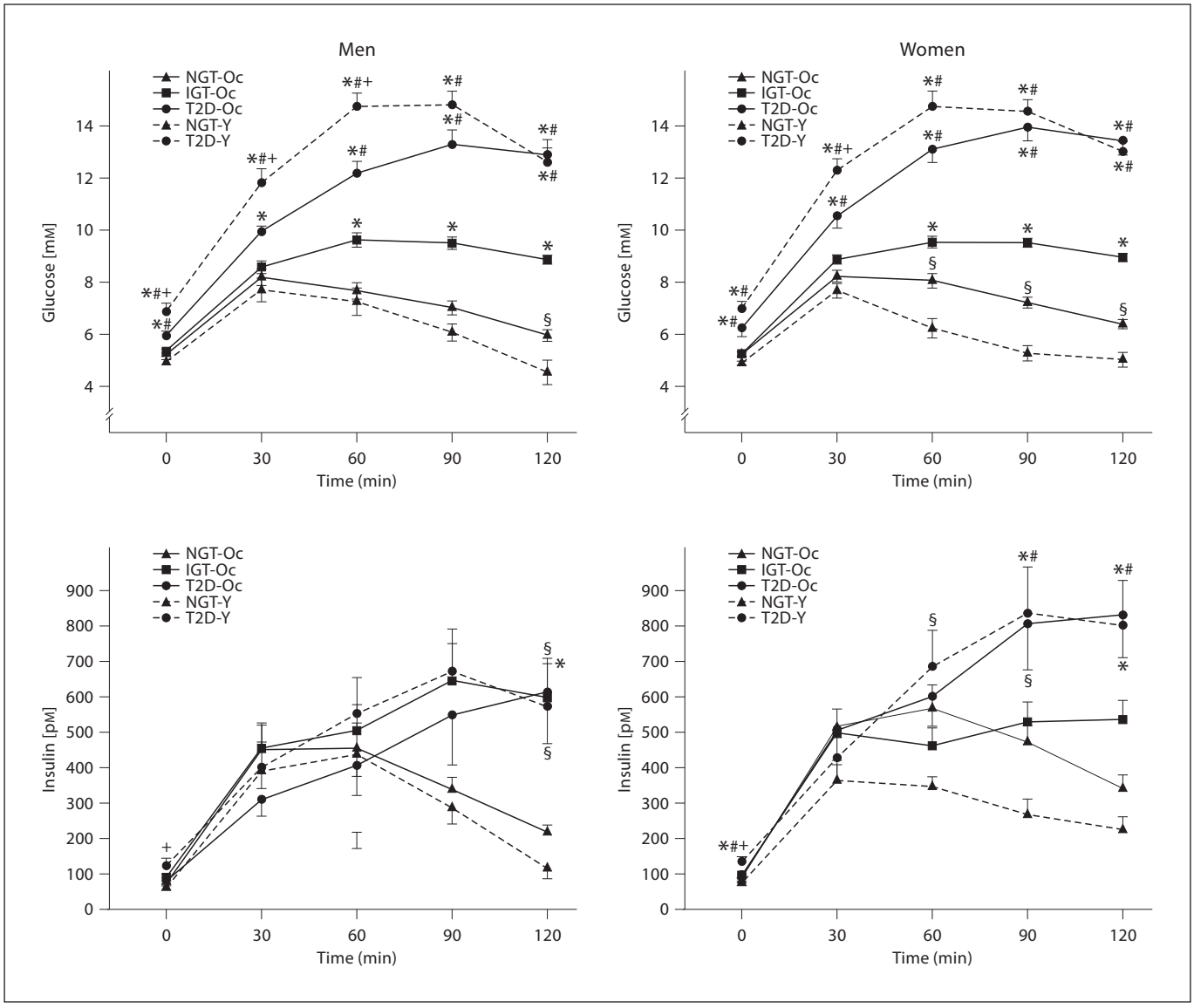

Fig. 1. Time course of plasma glucose and insulin in male and female octogenarians with NGT (NGT-Oc), IGT (IGT-Oc), type 2 diabetes (T2D-Oc) and corresponding middle-aged (younger) control groups with NGT (NGT-Y) and type 2 diabetes (T2D-Y) after standard OGTT. * $p \leq 0.05$ vs. NGT-Y and NGT-Oc ; # $p \leq 0.05$ vs. IGT-Oc; § $p \leq 0.05$ vs. NGT-Y; + $p \leq 0.05$ vs. T2D-Oc.

adiponectin. Therefore, no differences have been found for total adiponectin between male normoglycemic octogenarians and octogenarians with T2D.

Based on the observation that LMW adiponectin was lower in male as well as in female octogenarians with T2D, a multivariate analysis was performed in a dataset stratified by sex with all observation groups (octogenarians and middle-aged controls) and both sexes (table 4). The data were adjusted for sex, age, and BMI by using these parameters as covariates. Generally, the resulting multivariate model indicated a significant effect of all observation groups on serum adiponectin levels (Lawley-Hotellings's trace: $p = 0.047$). The subsequent univariate multi-range analysis with p values corrected for multiple tests (Bonferroni procedure) confirmed significantly lower levels of LMW adiponectin in octogenarians with T2D in comparison to NGT octogenarians and octogenarians with IGT (table 4).

Table 3. Fasting serum levels of total adiponectin and adiponectin isoforms in NGT-Oc, IGT-Oc, T2D-Oc, NGT-Y and T2D-Y

	Male octogenarians				Male younger controls		
	NGT-Oc (n = 24)	IGT-Oc (n = 24)	T2D-Oc (n = 10)	p	NGT-Y (n = 24)	T2D-Y (n = 14)	p
Total adiponectin	7.6 (6.3–8.8)	7.1 (5.8–8.4)	6.7 (4.8–8.7)	0.750	5.0 (3.4–6.6)	3.9 (2.5–5.2)	0.363
HMW adiponectin	3.3 (2.6–4.0)	3.1 (2.3–3.8)	3.3 (2.2–4.4)	0.898	2.0 (1.1–2.9)	1.4 (0.6–2.2)	0.390
MMW adiponectin	1.6 (1.3–2.0)	1.9 (1.5–2.2)	1.6 (1.1–2.2)	0.523	1.3 (0.9–1.7)	0.7 (0.4–1.1)	0.091
LMW adiponectin	2.7 (2.2–3.1)	2.1 (1.7–2.6)	1.8 (1.1–2.4)	0.049[1]	1.7 (1.2–2.3)	1.7 (1.3–2.2)	0.989
	Female octogenarians				Female younger controls		
	NGT-Oc (n = 38)	IGT-Oc (n = 39)	T2D-Oc (n = 19)	p	NGT-Y (n = 13)	T2D-Y (n = 19)	p
Total adiponectin	10.6 (9.3–11.9)	10.1 (8.9–11.4)	8.3 (6.5–10.1)	0.040[1]	7.1 (5.6–8.7)	5.8 (4.6–7.0)	0.258
HMW adiponectin	5.2 (4.4–6.0)	4.8 (4.0–5.6)	4.1 (2.9–5.2)	0.296	2.9 (2.0–3.9)	2.8 (2.0–3.5)	0.827
MMW adiponectin	2.5 (2.2–2.9)	2.2 (1.8–2.5)	2.2 (1.6–2.7)	0.283	1.5 (1.0–2.1)	1.1 (0.6–1.5)	0.277
LMW adiponectin	2.9 (2.5–3.2)	3.1 (2.8–3.5)	2.1 (1.5–2.6)	0.014[1, 2]	2.7 (2.1–3.2)	2.0 (1.5–2.4)	0.089

Data are presented as means [μg/ml] with 95% confidence interval; differences analyzed by ANCOVA with data adjusted for age and BMI; Bonferroni test $p \leq 0.05$: [1] T2D-Oc vs. NGT-Oc; [2] T2D-Oc vs. IGT-Oc.

Table 4. Results of a multivariate analysis of variance of the whole study population with age, sex, and BMI as covariates (effect of groups in the multivariate model: p = 0.047 by Lawley-Hotelling's trace)

	Groups					Univariate analysis	Post-hoc analysis with Bonferroni correction
	NGT-Oc (n = 62)	IGT-Oc (n = 63)	T2D-Oc (n = 29)	NGT-Y (n = 24)	T2D-Y (n = 33)	p	p
HMW adiponectin	3.9 (3.2–4.6)	3.7 (3.0–4.4)	3.3 (2.4–4.2)	3.9 (2.0–5.8)	3.5 (2.2–4.7)	0.626	n.s.
MMW adiponectin	1.9 (1.6–2.2)	1.8 (1.4–2.1)	1.7 (1.2–2.1)	2.2 (1.3–3.1)	1.7 (1.1–2.3)	0.402	n.s.
LMW adiponectin	2.6 (2.2–2.9)	2.6 (2.2–3.0)	1.8 (1.3–2.2)	2.7 (1.8–3.7)	2.3 (1.7–3.0)	0.002	0.003[1]

Data are presented as means [μg/ml] with 95% confidence interval adjusted for age, sex and BMI; Bonferroni test: [1] T2D-Oc vs. NGT-Oc and IGT-Oc.

Serum concentrations of total adiponectin and all adiponectin isoforms of male and female octogenarians with IGT were not statistically different from those of NGT octogenarians (table 3).

Correlation Analyses of Adiponectin with Clinical Data in Octogenarians

The results of the partial correlation analyses with data controlled for BMI are shown in online supplementary table D. An exceptionally high direct correlation of total adiponectin and all adiponectin multimer complexes with HDL cholesterol was found in both men and women. On the other hand, hip circumference was inversely correlated with total, HMW, and MMW adiponectin in men and women, and with LMW adiponectin in women only. Triglyceride levels revealed an inverse correlation with total, HMW, and MMW adiponectin and a direct correlation with the LMW/total adiponectin ratio in women.

Interestingly, LMW adiponectin, in contrast to HMW and MMW adiponectin, revealed a strong inverse correlation with fasting and 2-hour postchallenge glucose concentration in men and with 2-hour postchallenge glucose in women. Furthermore, the insulin levels 2 h after glucose load were inversely correlated with all adiponectin multimer isoforms in women.

Discussion

Aging is associated with an increased risk of insulin resistance and diabetes mellitus. The current study on a clinically well-characterized population of octogenarians with different insulin sensitivity demonstrates selectively lower serum LMW adiponectin in male and female octogenarians with T2D. Serum levels of LMW adiponectin were reversely correlated with plasma glucose level 2 h after oral glucose challenge. A tendency of lower LMW adiponectin has also been found in a middle-aged group of female individuals with T2D, but not in a corresponding middle-aged male group.

Serum levels of total adiponectin and its isoforms are known to be affected by aging [24, 25], sex [24–26], and insulin sensitivity status [24–26]. In accordance with earlier studies describing increased total adiponectin with age [24, 25], the present study revealed higher levels of total adiponectin and adiponectin isoforms in normoglycemic octogenarians of both genders. The age-dependent increase in total adiponectin was slightly more pronounced in men (77%) than in women (54%). While in men this increase was primarily due to the increase of HMW and LMW adiponectin, in women the increase of total adiponectin was mainly induced by the elevation of HMW and MMW adiponectin. As a result, the HMW/total adiponectin ratio increased in normoglycemic octogenarian men from 36.7 to 41.7% and in women from 43.1 to 47.5%, when compared with normoglycemic middle-aged individuals. Concurrently, the LMW/total adiponectin ratio decreased in normoglycemic octogenarian men from 39.1 to 31.1% and in women from 36.7 to 27.8% in comparison to middle-aged controls.

Although an increase in adiponectin levels with age has been reported by several studies [24, 25], the mechanisms for an age-related adiponectin increase are not fully understood. The effect of aging has been partially attributed to the reduction in the inhibitory effect of testosterone on adiponectin production with age. Notably, neither castration nor testosterone treatment modified the transcription activity of the adiponectin gene in adipocytes, suggesting that the regulation occurs at a post-transcriptional level [26]. Recent studies have demonstrated that the secretion of HMW adiponectin from adipocytes is much slower than that of LMW and MMW oligomer complexes, and that testosterone treatment leads to a further decrease in HMW adiponectin secretion [27]. These data suggest that different oligomeric complexes of adiponectin are released from adipocytes through at least two distinct secretory pathways [27]. Based on this, testosterone may selectively interfere with the HMW-specific mechanism of adiponectin secretion from the adipocyte. In line with this hypothesis, a more pronounced increase in HMW adiponectin in normoglycemic octogenarians could be explained in part by a selectively reduced inhibition of the testosterone-sensitive HMW secretory pathway. Very recently, an effect of female sex steroids on adiponectin oligomeric distribution in women has also been described [28]. While total, HMW, and MMW adiponectin was negatively associated with estradiol and progesterone, no association was observed for LMW adiponectin [28]. It is noteworthy that increasing concentrations of testosterone or estradiol influenced neither mRNA or protein expression nor adiponectin oligomer secretion in cultured preadipocytes [29].

Based on a differential hormonal background, sexual dimorphism in total, HMW, and MMW adiponectin has been reported in several studies on younger individuals [10, 11]. In extension of these data, the present study demonstrated gender differences also in octogenarians, in whom the hormonal background is expected to be more balanced. Women revealed significantly higher values for total, HMW, MMW, but not for LMW adiponectin. This observation is in accordance with Ebinuma et al. [11] who described the lack of gender difference for LMW adiponectin in a younger population, thereby supporting the hypothesis that, in contrast to higher molecular multimer complexes, sexual hormones might be less involved in the regulation of LMW adiponectin biosynthesis and secretion.

A growing number of studies indicate that the oligomer distribution of adiponectin is of greater relevance for its insulin-sensitizing activity than the plasma adiponectin concentration in total [7, 9, 30]. Earlier studies emphasized the importance of HMW adiponectin for the prediction and modulation of insulin resistance [12, 14]. The reduction in insulin sensitivity in patients with T2D was accompanied by a decline in HMW adiponectin accounting for the total adiponectin reduction [31]. Moreover, recent data demonstrated that the HMW/total adiponectin ratio was independent of total adiponectin related to the risk of T2D [14, 32] and coronary artery disease [10, 33]. Although the insulin-sensitizing properties of adiponectin have been mainly attributed to HMW adiponectin and the HMW/total adiponectin ratio [30], Fruebis et al. [6] demonstrated that different oligomers of adiponectin activated different signaling pathways in myotubes and isolated rat muscles. LMW adiponectin, but not the HMW isoform, induced phosphorylation of the AMPKα subunit and thereby its activation in myotubes and isolated rat muscles. In addition, the trimeric globular head of adipo-

nectin showed a much higher binding affinity than full-length adiponectin to Adipor1, the predominant form of adiponectin receptor expressed in skeletal muscle [34]. On the basis of these findings, it was supposed that the trimer is the most potent isoform mediating beneficial metabolic effects of adiponectin in skeletal muscle.

Interestingly, the present study demonstrated significantly lower serum LMW adiponectin in male and female octogenarians with T2D compared to normoglycemic octogenarians. In men with T2D, this situation resulted from a lack of age-induced LMW adiponectin increase, which was characteristic of normoglycemic men. In women, already middle-aged individuals with T2D exhibited a strong tendency for lower LMW adiponectin. This tendency was enhanced in octogenarian women with T2D. The pathogenetic mechanisms behind these changes are unknown. An interaction between hormonal shifts and the development of insulin resistance should be taken into consideration. One important point to consider is the specific metabolic situation of the investigated population of octogenarians. Clearly categorized by OGTT in terms of blood glucose and corresponding insulin curves, male and female octogenarians with T2D did not differ from octogenarians with NGT in BMI, WHR, blood pressure, HDL and total cholesterol. In accordance with earlier publications [35–38], the data of the present study revealed a highly significant direct correlation of all adiponectin multimer isoforms with HDL cholesterol in men as well as in women. Therefore, it was not surprising that the missing difference in HDL cholesterol between T2D octogenarians and the corresponding NGT group was accompanied by an absence of significant differences in HMW and MMW adiponectin. The nature of the close association between adiponectin and HDL cholesterol is not fully understood. However, in a rat model an increase in HDL cholesterol and a decrease in triglycerides were induced through the permanent activation of the AMPK pathway by an adenosine analogue [39]. This points, at least in part, to a direct effect of adiponectin on HDL metabolism.

The contribution of a selective decline in LMW adiponectin in octogenarians with T2D to the pathophysiology of diabetes has not been completely elucidated. Besides its peripheral action, adiponectin plays a significant role in the central regulation of food intake and energy expenditure. Very recently, Coope et al. [18] demonstrated in rats that intracerebroventricular infusion of adiponectin induced an anorexigenic effect that was mediated through hypothalamic AdipoR1 receptors. Notably, in both humans and rodents, only the trimeric and hexameric complexes of adiponectin are present in cerebrospinal fluid [20, 21]. The HMW oligomeric adiponectin is virtually undetectable in cerebrospinal fluid, perhaps due to the extremely large size of this complex (>500 kDa), which makes it difficult to translocate across the blood-brain barrier. Thus, the reduction in LMW adiponectin may be involved in a decline in insulin sensitivity in skeletal muscle and the central disinhibition of food intake promoting insulin resistance and obesity.

The increase in plasma adiponectin with age in normoglycemic individuals raised the question whether adiponectin might be a marker for longevity. This idea has been supported by recent studies showing an association of lower adiponectin levels with early onset and severity of coronary artery disease [40–42]. Low plasma levels of adiponectin were independently associated with increased intraventricular septum thickness, posterior ventricular wall thickness, and left ventricular mass index [43]. At the same time, evidence accumulates that increased adiponectin levels were closely associated with not only all-cause mortality but also coronary heart disease (CHD) mortality in either sex [44–46]. This association seems to be particularly strong in the elderly and in patients at high risk for CHD. A prospective study on more than 4,000 elderly men aged 60–79 confirmed the results of previous studies showing that high adiponectin levels were associated with significantly increased mortality in elderly patients with CHD [46]. Despite the rapidly accumulating literature, it is still uncertain whether adiponectin levels have any clinical significance for risk stratification in cardiovascular disease and aging or whether they just reflect the activation of complex and opposing underlying mechanisms. Therefore, the role of adiponectin as a marker for longevity should be discussed with caution.

In conclusion, the present study demonstrates significantly lower levels of plasma LMW adiponectin in male and female octogenarians with T2D in comparison to octogenarians with NGT, probably as a result of the interaction of aging and insulin resistance. At the same time, serum levels of HMW and MMW adiponectin isoforms were not significantly different. The pathophysiological importance of reduced serum LMW adiponectin in individuals with higher age and increased insulin resistance remains to be established.

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