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Adiponectin Oligomers in Human Serum during Acute and Chronic Exercise: Relation to Lipid Metabolism and Insulin Sensitivity

Abstract

Beneficial effects of physical exercise include improved insulin sensitivity, which may be affected by a modulated release of adiponectin, which is exclusively synthesized in white adipose tissue and mediates insulin sensitivity. Adiponectin circulates in three different oligomers, which also have a distinct biological function. We therefore aimed to investigate the distribution of adiponectin oligomers in human serum in relation to physical activity. Thirty-eight lean and healthy individuals were investigated. Seven healthy women and 8 healthy men volunteered to investigate the effect of chronic exercise, at 3 different time points with different training intensities. These individuals were all highly trained and were compared to a control group with low physical activity (n = 15). For studying acute exercise effects, 8 healthy men participated in a bicycle test. Adiponectin was determined by ELISA, oligomers were detected by non-denaturat-

ing western blot. Total adiponectin and oligomers were unchanged by acute exercise. LDL cholesterol was significantly lower in the chronic exercise group (p = 0.03). Total adiponectin levels and oligomers were not different between these two groups and were unaltered by different training intensities. However, total adiponectin and specifically HMW oligomers correlated with HDL cholesterol (r = 0.459; p = 0.009). We conclude that acute and chronic exercise does not directly affect circulating adiponectin or oligomer distribution in lean and healthy individuals. Whether such regulation is relevant in individuals with a metabolic disorder remains to be determined. However, our data suggest that adiponectin oligomers have distinct physiological functions *in vivo*, and specifically HMW adiponectin is closely correlated with HDL cholesterol.

Key words

Exercise · adiponectin · obesity · lipids · insulin sensitivity

Introduction

Physical activity has been shown to be associated with a reduced risk of cardiovascular disease and type 2 diabetes mellitus [8]. Substantial improvement of metabolic changes by physical exercise has been shown in individuals with existing metabolic syndrome. There is considerable evidence that beneficial effects depend at least in part on improvement of lipid metabolism and insulin resistance [3]. The endocrinological function of adipose tis-

sue has a major role in the development of insulin resistance. Adipocytes are apparently not only a reservoir for energy storage, but also an active endocrine organ [7]. The so called adipokines are well known to substantially affect glucose and lipid metabolism as well as central energy homeostasis [1, 35]. Adiponectin is exclusively synthesised in adipocytes and is an important mediator of glucose and lipid metabolism, which has been shown in animal and human studies [9, 19]. Adiponectin KO-mice have an impaired fatty acid clearance, increased tumor necrosis factor α

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levels, and insulin resistance under a high fat diet [15]. Interestingly, adiponectin levels are reduced in obese and insulin-resistant human subjects and animal models [31]. Despite the temptation to speculate that circulating adiponectin might be affected by the degree of physical activity, most studies have found that physical exercise has no influence on total adiponectin levels. Both short time exercise and long time physical training had no effect on total adiponectin plasma levels [10,13]. Rather controversially, a study by Jurimae et al. reported reductions directly after acute rowing. However, 30 min after the rowing, levels increased above resting values [11,14]. Kriketos et al. observed increased adiponectin levels following 2–3 bouts of exercise over 1 week, and these values remained elevated after 10 weeks of exercise [11,14].

Adiponectin is composed of a carboxyl-terminal globular domain and an amino-terminal collagenous domain [21,32]. Adiponectin belongs to the soluble collagen superfamily and has structural homology with collagen VIII, X, complement factor C1q [31], and the TNF family [16,21]. Members of this superfamily are known to form characteristic multimers. Indeed, gel filtration and velocity gradient studies revealed, that adiponectin is circulating in various different molecular weight species. The largest oligomer was shown to be more than several hundred kilodaltons in size [17,19]. Scherer et al. found that adiponectin from 3T3-L1 adipocytes forms trimers, hexamers and larger multimers [19]. Tsao et al. and Arita et al. analyzed multimer formation of adiponectin in serum by gel filtration chromatography and showed adiponectin to be separable into three species [25]. Waki et al. demonstrated with a SDS-PAGE under non-reducing and non-heat-denaturing conditions a relatively comfortable method, which is able to separate the multimers of adiponectin from various sources into the three circulating species, LMW (low molecular weight) trimers, MMW (middle molecular weight) multimers and HMW (high molecular weight) multimers [27]. Interestingly, biological activities of these different multimers appear to be multimer- and tissue-specific. The isolated globular domain of adiponectin stimulated fatty acid oxidation in skeletal muscle in an AMPK-dependent mechanism, whereas full length adiponectin improved insulin-mediated inhibition of hepatic glucose production [1,30]. While Waki et al. reported that the HMW oligomers activated AMP-activated protein kinase in hepatocytes [27], a recent report by Tsao et al. found that only trimers activate AMPK via STAT-dependent mechanisms in muscle cells. In this study, hexamers and HMW oligomers activated NF- κ B [26]. Differences in the tissue specific expression patterns of two adiponectin receptors may contribute to these divergent activities [29]. In humans, it has been described that HMW and total adiponectin correlate, to some extent, with insulin sensitivity [18,24].

Thus, there is considerable evidence that beneficial effects of physical activity on glucose and lipid metabolism might be mediated by adiponectin composition in the circulation, rather than total adiponectin levels. We therefore investigated the effect of short and long time physical activity on total adiponectin levels, adiponectin oligomers and correlated these factors to markers of glucose (fasting glucose, fasting insulin, HOMA-IR, QUICKI) and lipid metabolism (total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, free fatty acids).

Materials and Methods

Healthy individuals without current drug use were investigated. Impaired glucose metabolism was excluded by OGTT. The experimental protocol was approved by the Institutional Review Board, and all subjects gave written informed consent. Two major experiments were performed.

1) Acute physical exercise

In the first experiment, changes in adiponectin oligomers during acute exercise were investigated. Eight men (age: 35.2 ± 3.9 years, BMI: 24.2 ± 1.1) participated in this intervention. After an overnight fast, blood samples were taken after a rest of 20 minutes (time point 1). Afterwards, a bicycle test was accomplished. After 45 minutes of cycling at 50 W, intensity was increased by 25 W increments every 3 minutes until physical exhaustion. Mean highest bicycle test intensity was 209 ± 13 W. Additional blood samples were taken after 45 min (time point 2) and at the end of the bicycle test (time point 3).

2) Chronic physical exercise

Fifteen participants of the *real*-BERLIN-MARATHON (seven women and eight men) volunteered for this study. All participants reported regular physical activity with a minimum of 25 km running per week prior to initial testing. The $\dot{V}O_{2\max}$ was 42.9 ± 2.1 ml/kg·min for the female athletes and 48.3 ± 1.8 ml/kg·min for the male athletes. Blood samples were taken at 3 different time points, which was 6 weeks before a marathon (date 1) at a level of higher training amounts (50.3 ± 4.7 km running/week). Date 2 was two days after a marathon, and date 3 was 6 weeks after a marathon at a level of reduced training amounts (25.4 ± 4.0 km/week). All blood samples were taken in the morning after an overnight fast. The last training session with low intensity was allowed to be performed the day before blood samples were taken.

Additionally, untrained individuals were recruited from the MeSy-BePo (Metabolic Syndrome-Berlin Potsdam) study, which is an ongoing cross-sectional study with, currently, about 1500 individuals, investigating the pathogenesis of the metabolic syndrome. Fifteen subjects were matched for BMI, age and sex as a control group for the chronic physical exercise group. All individuals were healthy and did not exercise continuously. Physical activity was determined by a physical activity questionnaire, which revealed that none of the participants in the control group exceeded 1 hour of light physical activity per day (housework, car wash, etc.).

Laboratory parameters

After sampling in EDTA- or serum-tubes, blood was immediately chilled on ice, centrifuged and aliquots were frozen at -20°C until assayed. Blood samples were analyzed for glucose, insulin, CRP, FFAs (free fatty acids), cholesterol, LDL cholesterol, HDL cholesterol, triglycerides (TG), with COBAS MIRA from Roche (Lör-rach, Germany) (Intra-assay CV: glucose: 5.5%; insulin: 6.0%; CRP: 10.6%; FFA: 10.5%; cholesterol: 5.1%; HDL cholesterol: 5.4%; TG: 5.1%). Adiponectin concentrations were measured by immunosorbent assay (ELISA; Biovendor, Nasheville, TN, USA) (Intra-assay CV: 14.7%; Inter-assay CV: 7.3%).

Table 1 Biochemical characteristics during acute exercise; *p < 0.05 for comparison of time points

	Time 1	Time 2	Time 3
Glucose (mmol/l)	5.49 ± 0.08	5.68 ± 0.11	5.62 ± 0.19
Insulin (μU/l)	4.75 ± 0.63	3.78 ± 0.57	2.27 ± 0.37 *
HOMA-IR	1.17 ± 0.1	0.95 ± 0.1	0.57 ± 0.1 *
CRP (mg/l)	1.21 ± 0.32	1.22 ± 0.28	1.33 ± 0.20
Adiponectin (μg/ml)	7.43 ± 0.95	6.58 ± 0.88	8.08 ± 0.95
HMW %	13.22 ± 2.6	13.18 ± 0.6	13.97 ± 1.5
MMW %	44.20 ± 1.5	43.86 ± 2.7	44.14 ± 3.5
LMW %	42.50 ± 1.5	42.95 ± 2.5	41.89 ± 3.9
HMW absolute (μg/ml)	0.95 ± 0.1	0.87 ± 0.1	1.08 ± 0.1
MMW absolute (μg/ml)	3.34 ± 0.7	2.86 ± 0.3	3.56 ± 0.5
LMW absolute (μg/ml)	3.15 ± 0.5	2.85 ± 0.5	3.44 ± 0.6
Cholesterol (mmol/l)	4.75 ± 0.4	4.78 ± 0.3	5.43 ± 0.5 *
LDL cholesterol (mmol/l)	2.95 ± 0.4	2.89 ± 0.3	3.48 ± 0.5
HDL cholesterol (mmol/l)	0.88 ± 0.1	0.91 ± 0.1	1.01 ± 0.1 *
Triglyceride (mmol/l)	1.86 ± 0.3	1.97 ± 0.4	1.90 ± 0.3
FFA (mmol/l)	0.45 ± 0.1	0.50 ± 0.1	0.34 ± 0.1 *

Table 2 Biochemical characteristics during different training intensities; *p < 0.05 for comparison of time points

	Date 1	Date 2	Date 3
Glucose (mmol/l)	5.47 ± 0.1	5.71 ± 0.1	5.61 ± 0.1
Insulin (μU/l)	3.71 ± 0.3	4.04 ± 0.5	4.01 ± 0.4
CRP (mg/l)	1.96 ± 0.2	5.39 ± 0.6 *	1.48 ± 0.2
HOMA-IR	0.94 ± 0.1	1.04 ± 0.1	1.00 ± 0.05
Adiponectin (μg/ml)	14.33 ± 2.3	13.32 ± 2.0	13.78 ± 2.0
HMW %	15.09 ± 2.0	15.21 ± 1.8	15.67 ± 1.7
MMW %	48.28 ± 1.6	47.74 ± 2.1	45.50 ± 1.9
LMW %	36.63 ± 2.8	37.05 ± 3.1	38.83 ± 2.4
HMW absolute (μg/ml)	2.21 ± 0.4	2.02 ± 0.3	1.99 ± 0.3
MMW absolute (μg/ml)	6.90 ± 1.1	6.22 ± 1.0	6.54 ± 1.2
LMW absolute (μg/ml)	5.45 ± 1.0	4.84 ± 0.8	5.76 ± 0.8
Cholesterol (mmol/l)	5.04 ± 0.2	4.63 ± 0.1 *	5.10 ± 0.2
LDL Cholesterol (mmol/l)	2.43 ± 0.2	2.00 ± 0.1 *	2.49 ± 0.1
HDL Cholesterol (mmol/l)	1.98 ± 0.1	2.14 ± 0.1 *	2.00 ± 0.1
Triglyceride (mmol/l)	1.31 ± 0.4	0.81 ± 0.1 *	1.22 ± 0.3
FFA (mmol/l)	0.71 ± 0.1	0.55 ± 0.1	0.43 ± 0.1 *

Adiponectin oligomers were determined as previously described. Briefly SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Immunoblotting-SDS-PAGE was performed according to the standard Laemmli's method. Sample buffer for non-reducing, non-heat-denaturing conditions contained 3% SDS, 50 mM Tris-HCl pH 6.8 and 20% glycerol. For immunoblotting, proteins were separated by SDS-PAGE and transferred to nitrocellulose membranes. The membranes were washed with TBS-T (TBS, 0.1% Triton X-100) and then incubated with a polyclonal anti-

body (1 : 500; R & D Systems, Minneapolis, MN, USA) for 1 h at room temperature. After rigorous washing with TBS-T (3 · 10 minutes), the membranes were incubated with horseradish peroxidase-conjugated polyclonal goat anti-rabbit immunoglobulins (1 : 4000; DakoCytomation, Glostrup, Denmark) for 30 min at room temperature and then washed thoroughly. Bands were detected using HRP Western Blot detection system (Cell Signaling Technology, Inc., Beverly, MA, USA). Specificity of bands was shown by a competition. Therefore, the primary antibody was

pre-incubated overnight with an excess of antigen (R & D Systems), the protocol was otherwise unchanged.

Densitometry analysis of adiponectin oligomers was done with AIDA image analysis software (raytest GmbH, Straubenhardt, Germany). After adjusting for background activity, density of specific adiponectin oligomers bands were measured. Relative distribution of adiponectin oligomers was calculated by dividing band density through total density. Percentage of adiponectin oligomers was multiplied with total adiponectin levels to calculate absolute oligomer values.

Statistics

Given normal distribution of the data, which was analyzed by Shapiro-Wilk-test, subsequent analysis was performed by Student's *t*-test or repeated measures ANOVA, if various time points were compared. Correlations were analyzed in a group combined from individuals of the chronic exercise and the control group, and laboratory parameters of the chronic exercise group were taken from date 1 for these analyses.

Statistical calculations were performed with SPSS 11.5 (SPSS, Inc., Chicago, IL, USA). All values are given as mean and standard error. An alpha-error below 5% was considered as statistically significant.

Results

Acute exercise

During acute bicycle exercise, adiponectin levels did not change significantly. Total adiponectin levels were $7.43 \pm 0.95 \mu\text{g/ml}$ at rest, $6.58 \pm 0.88 \mu\text{g/ml}$ after 45 minutes at 50 W and $8.08 \pm 0.95 \mu\text{g/ml}$ at physical exhaustion ($p = 0.069$) (Fig. 1a).

We found no significant changes in adiponectin oligomers during acute cycling (Table 1). Between start, after 45 minutes at 50 W and at physical exhaustion, no significant differences could be observed. Also, the absolute amount of adiponectin oligomers did not change significantly (Fig. 1a). In contrast, insulin decreased during the bicycle test from $4.75 \pm 0.6 \mu\text{U/ml}$ to $2.27 \pm 0.3 \mu\text{U/ml}$ ($p < 0.001$), whereas glucose and CRP remained unchanged. Correspondingly, HOMA-IR (insulin·glucose/22.5) improved from 1.17 ± 0.1 at the beginning to 0.57 ± 0.1 at the end of the bicycle test ($p < 0.001$) (Fig. 1a). During the acute exercise, a significant increase in total cholesterol and HDL cholesterol was observed (Table 1), while a slight increase in LDL cholesterol failed to be significant. Triglycerides remained nearly unchanged, whereas FFA levels were decreased significantly from $0.45 \pm 0.1 \text{ mmol/l}$ to $0.34 \pm 0.1 \text{ mmol/l}$.

For estimating plasma volume shifts due to hemoconcentration by intensive exercise we measured plasma osmolality. However, no significant differences were found between the three time points ($p = 0.285$).

Chronic exercise

Adiponectin levels were unchanged by different training intensities (Fig. 1b). Adiponectin at higher training levels was $14.33 \pm 2.3 \mu\text{g/ml}$, $13.32 \pm 2.0 \mu\text{g/ml}$ two days after the marathon

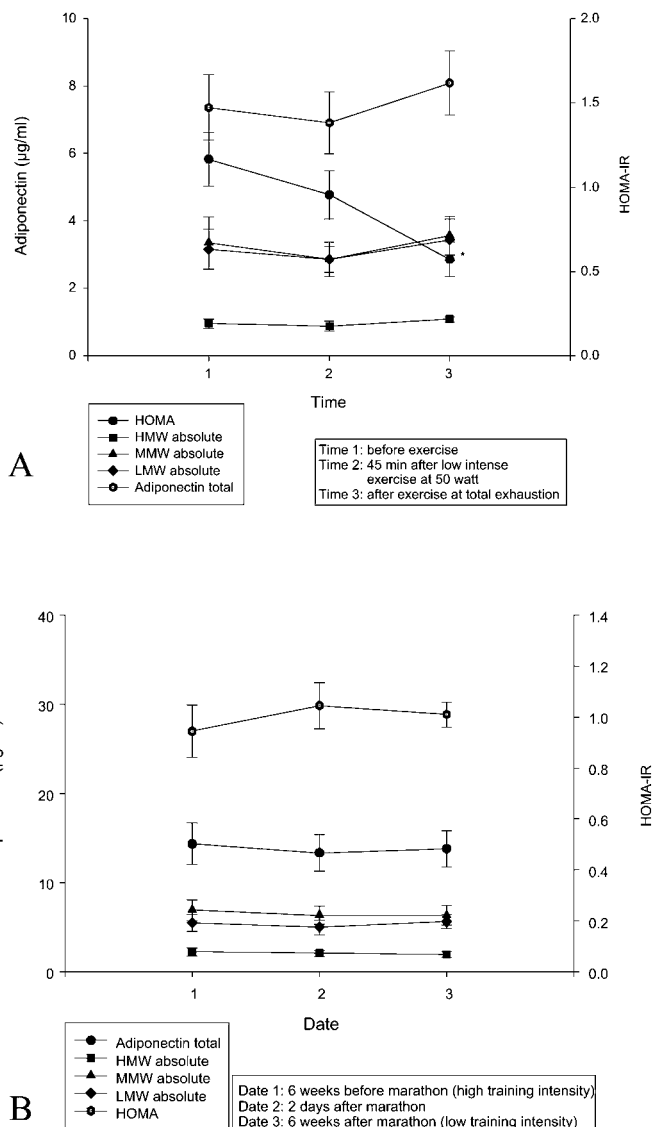


Fig. 1a and b a Adiponectin oligomers and insulin sensitivity during acute exercise. Changes of total adiponectin, adiponectin oligomers, and HOMA-IR during acute exercise. HOMA-IR decreased significantly ($p < 0.001$) after total physical exhaustion. b Adiponectin oligomers and insulin sensitivity during chronic exercise. Training amounts did not correlate with changes of adiponectin or its oligomers.

and $13.78 \pm 2.0 \mu\text{g/ml}$ 6 weeks after moderate training levels ($p = 0.751$). In addition, adiponectin oligomers were not affected by different training amounts (Fig. 1b). During chronic exercise at higher training intensity two days after the marathon and after 6 weeks of lower training amounts, no significant changes were observed (Table 2).

Body weight and BMI did not change significantly at the different training levels during the chronic exercise protocol. Also, glucose and insulin levels did not change (Table 2). Correspondingly, HOMA-IR was not changed during the marathon training (Fig. 1b). In contrast, CRP was significantly increased two days after the marathon ($5.39 \pm 0.6 \text{ mg/l}$) ($p < 0.001$) in relation to higher ($1.96 \pm 0.2 \text{ mg/l}$) and lower training amounts ($1.48 \pm 0.2 \text{ mg/l}$).

Table 3 Trained volunteers vs. untrained controls, which were matched for sex, age and BMI

	Untrained volunteers	Trained volunteers	P value
Number of individuals	15	15	–
Age (years)	45.0 ± 2.4	47.1 ± 2.1	matching variable
BMI (kg/m ²)	23.4 ± 0.5	22.9 ± 0.4	matching variable
Sex (female/male)	7/8	7/8	matching variable
Adiponectin (µg/ml)	11.42 ± 1.0	14.33 ± 2.3	0.26
HMW %	15.24 ± 3.1	15.09 ± 2.0	0.97
MMW %	51.10 ± 2.1	48.28 ± 1.6	0.32
LMW %	33.66 ± 3.4	36.63 ± 2.8	0.52
HMW absolute (µg/ml)	1.96 ± 0.5	2.21 ± 0.4	0.74
MMW absolute (µg/ml)	5.80 ± 0.5	6.90 ± 1.1	0.41
LMW absolute (µg/ml)	3.65 ± 0.4	5.45 ± 1.0	0.12
Cholesterol (mmol/l)	5.47 ± 0.3	5.04 ± 0.3	0.40
LDL cholesterol (mmol/l)	3.35 ± 0.2	2.43 ± 0.2	0.03
HDL cholesterol (mmol/l)	1.58 ± 0.1	1.98 ± 0.2	0.11
Triglyceride (mmol/l)	1.17 ± 0.1	1.31 ± 0.4	0.74
Glucose (mmol/l)	5.06 ± 0.2	5.47 ± 0.1	0.26
Insulin (µU/ml)	4.36 ± 0.6	3.71 ± 0.3	0.37
HOMA-IR	1.17 ± 0.3	0.94 ± 0.1	0.49
QUICKI	0.80 ± 0.0	0.75 ± 0.0	0.43
Physical activity (h/week)	1 ± 0.2	10 ± 0.5	< 0.001

Parameters of lipid metabolism were unaltered at different training levels, only FFA decreased significantly from date 1 with 0.71 ± 0.1 mmol/l to 0.43 ± 0.1 mmol/l at date 3 ($p = 0.002$). Only directly after the marathon at date 2, there was a significant decline in cholesterol-, LDL- and triglyceride levels and a significant increase in HDL cholesterol.

Metabolic characteristics of all 30 participants in the chronic exercise group and the control group, which were matched for BMI, age and sex for subsequent comparison, are shown in Table 3. However, as exact matches were not found, the chronic exercise group was slightly older and had a lower BMI (both not significant). Although exercising individuals had higher levels of HDL and lower cholesterol, HOMA-IR and insulin, these differences did not reach statistical significance. LDL was lower in trained individuals (at date 1) compared to controls ($p = 0.03$). Adiponectin levels were slightly higher in trained persons in contrast to controls, but again, this difference was not statistically significant. Also, no significant differences in adiponectin oligomers were found between highly trained individuals and controls.

In the whole group ($n = 30$), there was no correlation between insulin resistance (HOMA-IR) and total adiponectin or adiponectin oligomers (Fig. 2). A trend towards a positive correlation was found between HDL cholesterol and total adiponectin ($r = 0.339$; $p = 0.058$), which, however, closely failed to be significant. Significant correlations were found between HDL, relative HMW ($r = 0.225$; $p = 0.022$) and HMW absolute ($r = 0.459$; $p = 0.009$) (Fig. 2). In contrast, the other oligomers, MMW and LMW, did

not correlate with HDL or any other parameter of lipid or glucose metabolism.

Discussion

This study demonstrated that acute exercise improved insulin sensitivity, thus confirming results of previous studies. In addition, parameters of lipid and glucose metabolism were found to be favorable in chronically exercising individuals compared to those with a sedentary lifestyle, despite matching for BMI, age and sex and despite investigating healthy young individuals. No direct effect of acute or sustained physical activity was found on circulating adiponectin or its oligomer distribution. In addition, we were unable to identify an association between insulin sensitivity and any specific adiponectin oligomer. In contrast, HDL levels correlated with HMW adiponectin. Our data suggest that adiponectin-oligomers might have distinct physiological functions *in vivo*, but do not mediate the favorable effects of physical exercise.

Physical exercise is associated with a reduced risk for the development of obesity-associated co-morbidities like type 2 diabetes mellitus [8] and reduces the mortality risk of individuals with impaired glucose tolerance to that of healthy persons [5]. The improvement of insulin sensitivity by physical activity has been proposed as a possible mechanism of these effects [12]. From a mechanistic point of view, adipokines have been identified as potential mediators between obesity and insulin sensitivity. Despite this tentative link, we were unable to find an associ-

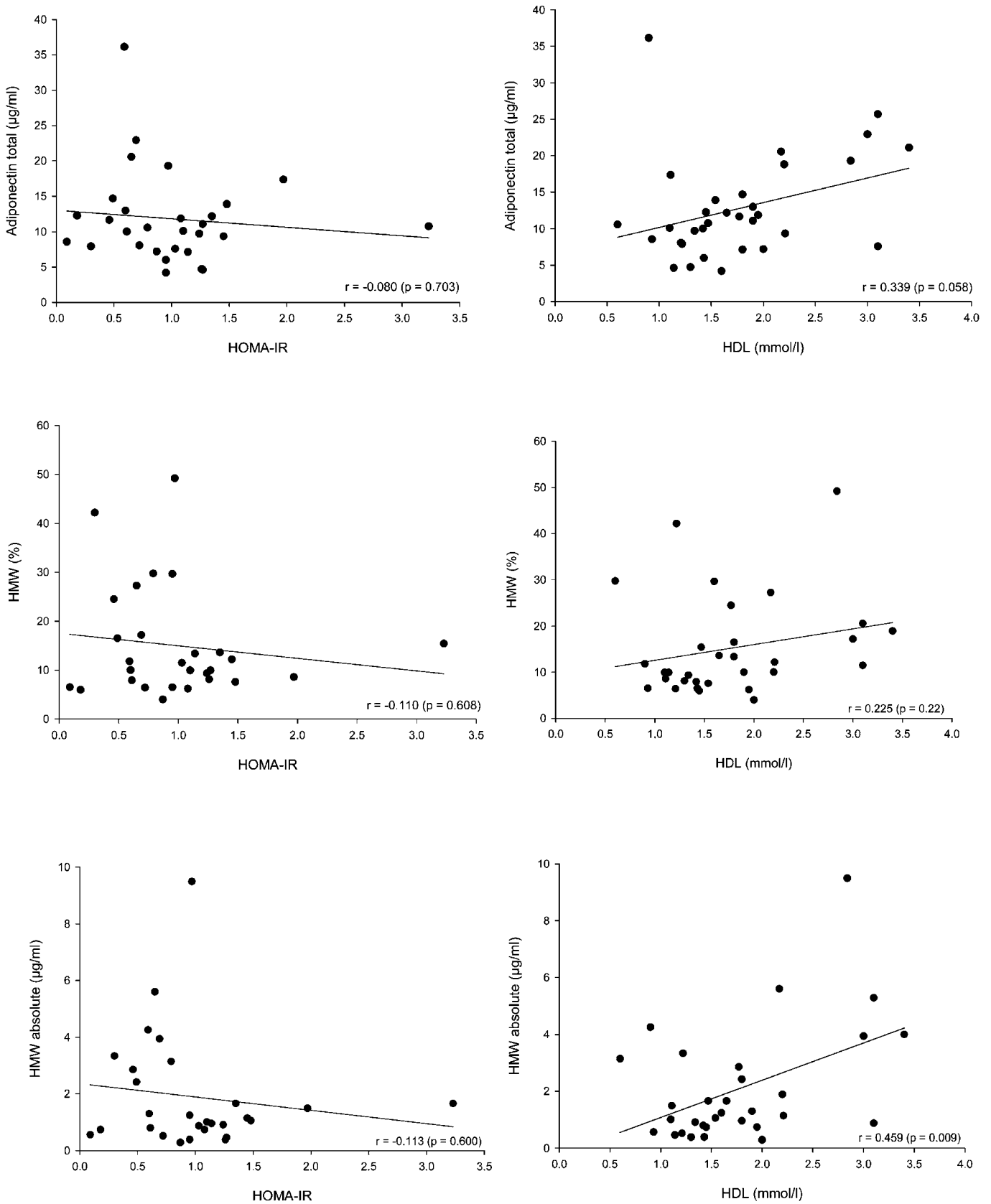


Fig. 2 Correlation between total adiponectin, relative HMW, HMW absolute, HDL, and HOMA-IR. Correlation between total adiponectin and HMW with HDL cholesterol, respectively. In contrast no substantial correlation was identified between insulin resistance and total adiponectin or adiponectin oligomers.

ation between exercise and circulating levels of adiponectin. With respect to total adiponectin levels, our results basically confirm those of other studies, which were also unable to identify a relationship between total adiponectin and the degree of acute physical activity [6,10,13,33]. However, one study in highly trained rowers found a decrease of adiponectin directly after a maximal exercise, while adiponectin increased about half an hour after the end of exercise [11]. Thus, the effects of acute sport might depend on the baseline cardiovascular condition, the degree of exercise, the precise type of exercise and the time point of adiponectin measurement. Recent studies suggested that the biological activity of adiponectin may be multimer- and tissue-specific. Waki and co-workers reported that the HMW oligomers activated AMP-activated protein kinase in hepatocytes [27], while another study described that only trimers activate AMPK via STAT-dependent mechanisms in muscle cells. In the same study hexamers and HMW oligomers activated NF- κ B [26], which was suggested to mediate negative effects on insulin sensitivity [34]. In patients with type 2 diabetes, it has been described that HMW adiponectin has a better correlation to insulin sensitivity than total adiponectin [18,24]. Although it was tempting to speculate that differences in the distribution of adiponectin oligomers might partially explain beneficial effects of physical activity on insulin sensitivity, we were unable to confirm such a relationship and found no effects of acute exercise on adiponectin oligomers. Thus, the beneficial acute effects of physical activity with respect to insulin sensitivity are unlikely to be mediated by changes of adiponectin oligomers. Significant changes in lipid metabolism by acute exercise were observed only for HDL, total cholesterol and FFAs, which confirmed previously published results [22]. Plasma volume shifts are well known to occur during acute exercise and may have biased our results. We therefore determined plasma osmolality, which did not change between the different time points of acute exercise, suggesting that the effect of any plasma volume shifts is likely to be small. However, although unchanged plasma osmolality may provide some rationale that plasma volume shifts are unlikely to have biased the results considerably, we are aware that plasma osmolality is no perfect control for plasma volume shifts. Since hematocrit was not determined within this study, potentially minor effects of plasma volume shifts cannot be completely ruled out.

Interestingly, individuals with sustained physical exercise were more insulin sensitive, had lower total cholesterol and LDL levels and higher HDL levels compared to sedentary controls, although this difference was only significant with respect to LDL. These findings are striking taking into account that the controls were comparable to the chronic exercise group with respect to age, sex and BMI. However, although BMI is comparable, body composition is likely to be different between exercising and sedentary individuals, which might partially explain the metabolic differences. As in the acute exercise group, we found no effect of chronic exercise on the distribution of adiponectin oligomers and no influence of training intensities was observed in the higher trained individuals. Interestingly, Tonelli et al. showed that HMW correlated positively with hepatic insulin sensitivity in patients with type 2 diabetes after treatment with thiazolidinediones [24]. In slight contrast to this study, we did not find a correlation between HMW adiponectin and insulin sensitivity in the

combined control and chronic exercise group. Although, this cohort comprised 30 individuals and is thus not extraordinary large, its size is still comparable to most presently published studies and this result was therefore somewhat surprising. Beside the fact that the sample size may have been still too small to detect significant effects, insulin sensitivity was determined in our cohort by HOMA-IR. Given a significant correlation between HOMA and M-values from euglycemic-hyperinsulinemic clamps ($r = -0.512$), we demonstrated the validity of HOMA-IR in our region. However, HOMA-IR is still different compared to euglycemic clamp, which may have considerable influence on the results. In addition, variability of insulin resistance was quite low in our cohort, as we investigated primarily young and healthy individuals. Thus, inclusion of obese patients with metabolic disorders might modify the picture. We cannot exclude that adiponectin oligomers might have an important role in the mediation of exercise-induced effects in obesity or impaired glucose or lipid metabolism. In addition, the mechanisms which influence adiponectin concentrations after chronic or acute exercise might be different and could also depend on factors, like type of exercise or the time point when adiponectin concentrations were measured. Despite these limitations, our data suggest that changes of adiponectin oligomers are probably not responsible for the beneficial effects of sustained physical exercise on lipid and glucose metabolism.

Plasma adiponectin levels are known to be reduced in states of insulin resistance compared with healthy controls [28] and recent studies demonstrated a relationship between adiponectin and fat metabolism [4,23]. We indeed found a positive correlation between adiponectin and HDL cholesterol in the combined control and chronic exercise group. Similar results were reported by Schulze and co-workers, who also reported a relation between HDL cholesterol and total adiponectin levels [20]. With respect to oligomers, in our study, only HMW was directly correlated to HDL cholesterol, suggesting that this oligomer is primarily driving this relationship. Thus, there is some evidence that the HDL level and HMW adiponectin are interrelated, although the precise nature of this interplay needs to be determined in subsequent studies.

In summary, acute and chronic exercise did not directly affect circulating adiponectin or oligomer distribution in lean and healthy individuals. Whether such regulation is relevant in obese individuals or patients with an existing metabolic disorder is unclear and remains to be determined. Our data suggest that adiponectin oligomers have distinct physiological functions *in vivo* and HMW adiponectin may affect circulating HDL cholesterol.

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