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Association of adiponectin and leptin with relative telomere length in seven independent cohorts including 11,448 participants

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Abstract Oxidative stress and inflammation are major contributors to accelerated age-related relative telomere length (RTL) shortening. Both conditions are strongly linked to leptin and adiponectin, the most prominent adipocyte-derived protein hormones. As high leptin levels and low levels of adiponectin have been implicated in inflammation, one expects adiponectin to be positively associated with RTL while leptin should be negatively associated. Within the ENGAGE consortium, we investigated the association of RTL with adiponectin and leptin in seven independent cohorts with a total of 11,448 participants. We performed partial correlation analysis on Z-transformed RTL and LN-transformed leptin/adiponectin, adjusting for age and sex. In extended models we adjusted for body mass index (BMI) and C-reactive protein (CRP). Adiponectin showed a borderline significant association with RTL. This appeared to be

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determined by a single study and when the outlier study was removed, this association disappeared. The association between RTL and leptin was highly significant ($r = -0.05$; $p = 1.81 \times 10^{-7}$). Additional adjustment for BMI or CRP did not change the results. Sex-stratified analysis revealed no difference between men and women. Our study suggests that high leptin levels are associated with short RTL.

Keywords Telomere length · Adipocytokines · Leptin · Oxidative stress · Inflammation

Introduction

Both leptin and adiponectin are among the most prominent adipocyte-derived protein hormones. Their origin in adipocytes and ability to affect the expression of various markers of systemic inflammation has led to the notion of both protein hormones as adipocytokines [1].

High leptin levels have been implicated in contributing to inflammation, insulin resistance, glucose intolerance and atherosclerosis [2]. Low levels of adiponectin, on the other hand, have been linked to inflammation, insulin resistance, impaired endothelium-dependent vasodilatation, elevated systemic blood pressure and further deteriorating effects on cardiovascular and metabolic health [3]. Interestingly, for both adipocytokines, impaired efficacy at the level of the receptors have been considered to be a mechanism potentially akin to that seen under the condition of sustained elevated insulin levels (insulin resistance) [4].

Telomeres are tandem repeats of long hexamers (TTAGGG) at the end of chromosomes that protect against spontaneous DNA damage and thus preserve genomic integrity [5, 6]. Oxidative stress and inflammation are major causative contributors to accelerated relative telomere length (RTL) shortening [7–10]. Telomere length has been associated with multiple disease, including cardiovascular diseases, one of the major causes of mortality [11–13]. As described above both adipocytokines also have a documented role in cardiovascular diseases, however for adiponectin the directionality of this association is unclear [14]. In this study we aim to elucidate the causal pathway of the pathological process. Given the associations of the adipocytokines with inflammation and oxidative stress, one would expect that adiponectin should be positively associated with RTL while leptin should be negatively associated.

Only a limited number of studies have investigated the association of adipocytokines with RTL and have yielded conflicting findings. Up to now, six studies have been published on the relation between RTL and leptin with sample sizes varying from 317 to 2,721 subjects [15–20]. An inverse association was found in two studies [15, 19] while two did not find evidence for a significant association [16, 20]. One study found borderline significant evidence for a positive association [17]. The last study found borderline significant evidence for a positive association when

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Table 1 Description of study populations

Population	Country	Design	Ethnicity	Exclusions[a]
ERF	Netherlands	Family-based	Caucasian	None
KORA F3	Germany	Population-based	Caucasian	None
KORA F4	Germany	Population-based	Caucasian	None
LLS	Netherlands	Family-based	Caucasian	None
TwinsUK	United Kingdom	Twin registry	Caucasian	None
SAPHIR	Austria	Population-based	Caucasian	Severe obesity, established coronary heart disease, congestive heart failure, valvular heart disease, chronic alcohol consumption, drug abuse, pregnancy
Finnish Twins	Finland	Twin registry	Caucasian	None

[a] Exclusions: study-wide exclusion criteria

adjusting for percentage body fat [18]. There have been 3 previous studies on the association between RTL and adiponectin, including 193–570 subjects [16, 20, 21] with one showing a positive association [21]. In the present study we aimed to investigate the association of RTL with adiponectin and leptin in seven independent cohorts with a total of 11,448 participants.

Methods

Study populations

The study protocol was approved by the medical ethics boards of the participating centers. Written informed consent was obtained from all included study participants. A summary of the design of the included study populations is provided in Table 1. More detailed information is provided below.

The Erasmus Rucphen Family (ERF) study is a cross-sectional cohort including 3,000 living descendants of 22 couples who had at least 6 children baptized in the community church around 1850–1900. The participants are not selected on any disease or other outcome. Details about the genealogy of the population have been described elsewhere [22, 23].

The KORA (Cooperative Health Research in the Region of Augsburg) study is a series of independent population-based epidemiological surveys and follow-up studies of participants living in the region of Augsburg, Southern Germany [24]. All survey participants are of German nationality, identified through the registration office. Informed consent has been given by all participants. The present study includes data of the KORA F3 (2004/2005) survey which is a follow-up study of the KORA S3 survey (1994/1995), as well as data of the KORA F4 (2006–2008) study which is a follow-up study of the KORA S4 survey (1999–2001). All KORA F4 participants were fasting. In KORA F3, adjustment for fasting status was made.

For the Leiden Longevity Study (LLS), long-lived siblings of Dutch descent were recruited together with their offspring and the partners of thereof. Families were included if at least two long-lived siblings were alive and fulfilled the age criterion of 89 years or older for males and 91 years or older for females, representing <0.5 % of the Dutch population in 2001 [25]. In total, 944 long-lived proband siblings from 421 families with a mean age of 94 years (range 89–104), 1,671 offspring (61 years, 39–81), and 744 partners (60 years, 36–79) were included in the study. DNA from the LLS was extracted from samples at baseline using conventional methods [26]. For the current analysis only the offspring and their partners were used.

The TwinsUK cohort (www.twinsuk.ac.uk) is an adult twin British registry shown to be representative of singleton populations and the United Kingdom population [27]. A total of 6,038 twins with RTL measurement were included in the analysis. The age range of the TwinsUK cohort was 16–99 years.

The Salzburg Atherosclerosis Prevention program in subjects at High Individual Risk Study (SAPHIR) is an

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observational study in a healthy working population conducted in the years 1999–2002 involving 1532 healthy unrelated subjects with RTL measurement available: 539 females aged 42–67 years and 993 males aged 39–66. All subjects were recruited by health-screening programs in companies in and around the Austrian city Salzburg (1999–2002) [28]. At baseline, all study participants were subjected to a comprehensive screening examination with a detailed personal and family history assessed via standardized questionnaires and a physical examination. Subjects suffering from severe obesity (BMI >40 kg/m^2), established coronary artery disease, congestive heart failure, valvular heart disease, chronic alcohol consumption (more than three drinks per day), drug abuse or pregnancy were excluded.

The Finnish twin participants were recruited from two population-based longitudinal studies, FinnTwin16 and FinnTwin12, each consisting of five consecutive birth cohorts (1975–1959, n = 5,601 subjects in FinnTwin16 and 1983–1987, n = 5,184 in FinnTwin12, respectively) [29]. All pairs were of Caucasian origin. Except for one obese male subject who had recently developed type 2 diabetes and used insulin, the subjects were healthy and did not take any medications. Zygosity was confirmed by genotyping of ten informative genetic markers [30]. For the current study only those individuals with measurements on both telomere length and adipokines were included (n = 190). They had taken part in a sub-study of metabolic factors related to obesity, and were representative of the distribution of BMI in the two twin cohorts. No further exclusion criteria were used.

Relative TL, adiponectin and leptin measurements

Details on the measurements of adiponectin, leptin and RTL for the individual cohorts have been published elsewhere and are provided for this paper in the supplementary material. In all studies RTL was measured in leukocytes by qPCR [31]. For 4 out of the 7 studies RTL was determined in one central laboratory according to common protocol. Adiponectin was available in all cohorts, while leptin was not available in SAPHIR.

Data analysis

In order to standardize RTL measurements across cohorts a Z-transformation was applied. Adiponectin and leptin levels were transformed using a LN-transformation. Samples deviating more than 4 standard deviations from the mean were removed (Supplementary Table 1). We performed partial correlation analysis, adjusting for age and sex, and, if necessary, for family relationships and/or fasting status. In the extended model we additionally adjusted for body mass index (BMI) or C-reactive protein (CRP). We also performed sex-specific analyses, thus resulting in a Bonferroni corrected p-value threshold for significance of 0.006 (=0.05/9). For the association of leptin and adiponectin with telomere length we finally created age and sex adjusted telomere length quartiles and associated these with leptin and adiponectin in the whole population.

We further performed power analysis using the 'pwr' library in R [32] to determine the minimal sample size required to detect a correlation between 0.02 and 0.10 at a significance level threshold of 0.006 for power values ranging from 0.5 to 0.9.

Results

The general characteristics of the study populations are depicted in Table 2 stratified for men and women. Most cohorts had a mean age around 50 years, except for Finnish Twins in which the mean age was 28 years at the time of assessment. Most cohorts also had an approximately equal number of men and women, except for TwinsUK which includes only women.

Power analysis showed that with the current sample size of over 10,000 subjects we had a power of over 80 % to detect correlation coefficients of 0.04 or higher (Supplementary Figure 1).

Table 3 contains the partial correlation analysis of RTL and BMI. We observed a nominally significant correlation between BMI and RTL in the total population (r = −0.02, $p = 0.021$). We did not observe a significant correlation between CRP and RTL (r = −0.01, $p = 0.444$, Supplementary Table 2).

Table 4 shows the partial correlation analysis of RTL and adiponectin. In the overall meta-analysis, we observed a nominally significant correlation between RTL and adiponectin (r = 0.02, $p = 0.028$). There was significant evidence for heterogeneity ($I^2 = 69.8$ %). The high heterogeneity was caused by one outlying study (ERF). In a sensitivity analysis we removed ERF from the meta-analysis, which completely resolved the heterogeneity and resulted in a loss of significance (r = 0.01, $p = 0.592$). Additionally adjusting for BMI (r = 0.02, $p = 0.079$) or CRP (r = 0.02, $p = 0.047$) did not change the results (Supplementary Table 3). The association of quartiles of RTL with adiponectin also revealed no association (Supplementary Table 4).

The results of the partial correlation analysis of RTL and leptin are depicted in Table 5. We observed a weak, but highly significant, correlation between RTL and leptin in the overall meta-analysis (r = −0.05, $p = 1.81 \times 10^{-7}$). No heterogeneity was observed ($I^2 = 0$ %). When

Table 2 General characteristics of study populations

	Men			Women		
	n	Mean	SD	n	Mean	SD
Telomere length (t/s ratio)						
ERF	1,230	1.74	0.35	1,539	1.81	0.36
KORA F3	1,517	1.69	0.28	1,607	1.75	0.29
KORA F4	1,456	1.79	0.32	1,568	1.90	0.32
LLS	1,043	1.43	0.25	1,262	1.48	0.26
TwinsUK	NA	NA	NA	1,428	3.50	0.63
SAPHIR	982	0.84	0.16	530	0.85	0.16
Finnish Twins	107	1.29	0.28	83	1.24	0.23
Adiponectin (mg/L)						
ERF	904	8.06	4.13	1,188	12.36	5.79
KORA F3	1,535	8.67	3.66	1,624	12.14	4.57
KORA F4	581	8.95	5.38	550	14.56	7.35
LLS	1,036	4.81	2.19	1,207	7.23	3.12
TwinsUK	NA	NA	NA	1,185	7.82	3.65
SAPHIR	982	7.01	3.15	530	10.85	4.67
Finnish Twins	107	2.30	0.77	83	3.22	1.02
Leptin (ng/mL)						
ERF	687	24.91	29.38	905	74.85	65.35
KORA F3	800	9.53	9.41	825	30.76	26.12
KORA F4	1,467	9.58	11.37	1,584	28.18	23.70
LLS	1,042	9.66	8.98	1,206	26.19	18.28
TwinsUK	NA	NA	NA	1,428	16.89	11.52
SAPHIR	NA	NA	NA	NA	NA	NA
Finnish Twins	107	5.58	4.69	83	20.96	15.89
Age (year)						
ERF	1,230	50.17	14.65	1,539	49.44	15.05
KORA F3	1,545	57.73	13.15	1,639	57.10	12.66
KORA F4	1,486	56.64	13.41	1,594	55.51	13.14
LLS	1,063	59.87	6.85	1,288	58.60	6.76
TwinsUK	NA	NA	NA	1,428	48.71	13.48
SAPHIR	985	48.85	5.46	539	55.63	4.18
Finnish Twins	107	28.25	1.86	83	28.08	1.60
BMI (kg/m2)						
ERF	1,191	27.24	4.13	1,472	26.65	5.10
KORA F3	1,531	28.01	3.86	1,626	27.27	5.16
KORA F4	1,480	27.93	4.19	1,583	27.33	5.32
LLS	904	25.77	3.05	1,101	25.10	3.94
TwinsUK	NA	NA	NA	1,426	24.85	4.41
SAPHIR	984	26.91	3.70	539	26.59	4.75
Finnish Twins	107	24.92	3.72	83	24.19	5.23
CRP (mg/L)						
ERF	849	3.53	10.50	1,131	3.92	8.13
KORA F3	144	4.06	5.72	117	5.06	10.67
KORA F4	1,477	2.40	5.18	1,582	2.61	5.41
LLS	1,029	2.11	2.89	1,234	2.53	3.66
TwinsUK	NA	NA	NA	1,250	3.02	4.44
SAPHIR	983	0.26	0.73	538	0.35	0.51
Finnish Twins	107	0.75	1.03	83	2.31	3.36

NA not available

removing the cohort with the strongest association (KORA F4) from the analysis, the correlation did not change ($r = -0.04$, $p = 1.17 \times 10^{-3}$). Additionally adjusting for BMI ($r = -0.04$, $p = 1.93 \times 10^{-5}$) or CRP ($r = -0.06$, $p = 2.10 \times 10^{-8}$) did not change the results (Supplementary Table 5). The multivariate analysis shows that leptin explains 0.16 % of the telomere length variance. The sex-stratified analysis also showed very similar correlations for men and women ($r = -0.06$ and $r = -0.05$ respectively), which indicates that the association between RTL and leptin is not sex-specific. The association of quartiles of RTL with leptin showed a linear decline of leptin levels with increasing telomere length (p-value for trend $= 2.35 \times 10^{-7}$; Supplementary Table 6).

Discussion

In this study including data of 11,448 subjects derived from population-based studies, we found evidence for a significant negative association between RTL and serum leptin levels. The relationship was independent of age, sex, BMI and CRP. However, no relationship was found between RTL and adiponectin. A nominally significant association of RTL with BMI was also observed.

The correlation between leptin and RTL, adjusted for age and sex was small but highly significant ($r = -0.05$, $p = 1.81 \times 10^{-7}$). Based on the assumption of an association between high leptin levels and inflammation [2], we observed an inverse association between leptin and RTL. The current study is the largest study on the association between RTL and leptin to date with 10,093 subjects studied compared to the previous studies with sample size ranging from 317 to 2,721 subjects [15–20]. Two out of the six previously performed studies on the association between leptin and RTL also found an inverse association only in women [15, 19]. The present study therefore provides a strong confidence in the observed association and elucidates the association in both men and women.

High leptin levels have been implicated in contributing to inflammation, to insulin resistance, glucose intolerance and atherosclerosis [2]. Furthermore, leptin is an important mediator in stress-induced cardiovascular activity mainly by raising circulating catecholamine concentrations and by

Table 3 Correlation between telomere length and BMI

Study	Total				Men				Women			
	n	r	SE	*p* value	n	r	SE	*p* value	n	r	SE	*p* value
ERF	2,663	0.003	0.02	0.891	1,191	−0.01	0.03	0.664	1,472	−0.01	0.03	0.749
KORA F3	3,095	−0.01	0.02	0.674	1,501	0.001	0.03	0.984	1,594	−0.02	0.03	0.415
KORA F4	2,998	−0.07	0.02	1.20×10^{-4}	1,442	−0.09	0.03	3.90×10^{-4}	1,556	−0.06	0.03	0.027
LLS	1,949	−0.003	0.02	0.882	883	0.01	0.03	0.776	1,066	−0.01	0.03	0.718
TwinsUK	1,426	−0.02	0.03	0.450	0	NA	NA	NA	1,426	−0.02	0.03	0.450
SAPHIR	1,520	−0.01	0.03	0.607	982	−0.04	0.03	0.181	538	0.02	0.04	0.591
FTC	190	0.06	0.07	0.423	107	0.01	0.10	0.904	83	0.10	0.11	0.373
Meta-analysis	13,841	−0.02	0.01	0.021	6,106	−0.03	0.01	0.020	7,735	−0.02	0.01	0.084

NA: not available

Table 4 Correlation between telomere length and adiponectin

	Total				Men				Women			
Study	n	r	SE	*p* value	n	r	SE	*p* value	n	r	SE	*p* value
ERF	2,092	0.09	0.02	6.38×10^{-5}	904	0.07	0.03	0.043	1,188	0.11	0.03	1.03×10^{-4}
KORA F3	3,120	0.02	0.02	0.171	1,515	0.001	0.03	0.963	1,605	0.05	0.02	0.056
KORA F4	1,116	−0.04	0.03	0.178	574	−0.07	0.04	0.082	542	−0.005	0.04	0.914
LLS	2,233	0.001	0.02	0.944	1,018	0.02	0.03	0.034	1,215	−0.02	0.03	0.109
TwinsUK	1,185	0.002	0.03	0.942	NA	NA	NA	NA	1,185	0.00	0.03	0.942
SAPHIR	1,512	0.02	0.03	0.449	982	0.02	0.03	0.581	530	0.02	0.04	0.700
FTC	190	−0.08	0.07	0.247	107	0.02	0.10	0.815	83	−0.25	0.11	0.024
Meta-analysis	11,448	0.02	0.01	0.028	5,100	0.02	0.01	0.121	6,348	0.02	0.01	0.068

NA not available

Table 5 Correlation between telomere length and leptin

	Total				Men				Women			
Study	n	r	SE	*p* value	n	r	SE	*p* value	n	r	SE	*p* value
ERF	1,592	−0.03	0.03	0.201	687	−0.03	0.04	0.368	905	−0.05	0.03	0.156
KORA F3	1,613	−0.03	0.02	0.247	797	−0.01	0.04	0.816	816	−0.05	0.04	0.170
KORA F4	3,010	−0.08	0.02	2.84×10^{-5}	1,443	−0.11	0.03	3.83×10^{-5}	1,567	−0.04	0.03	0.087
LLS	2,260	−0.05	0.02	0.013	1,031	−0.05	0.03	0.090	1,229	−0.05	0.03	0.070
TwinsUK	1,428	−0.05	0.03	0.054	NA	NA	NA	NA	1,428	−0.05	0.03	0.054
SAPHIR	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
FTC	190	−0.03	0.07	0.680	107	−0.11	0.10	0.262	83	0.09	0.11	0.422
Meta-analysis	10,093	−0.05	0.01	1.81×10^{-7}	4,065	−0.06	0.02	7.38×10^{-5}	6,028	−0.05	0.01	3.26×10^{-4}

NA not available

stimulating heart rate and arterial blood pressure [1, 33]. Though the percentage of variance explained is small (~0.16 %), the observed association between leptin and telomere length is interesting, due to the biological link with both inflammation and oxidative stress of both variables.

The findings on adiponectin were not consistent over studies with evidence for substantial heterogeneity. Removing the outlier study (ERF) led to a non-significant association. There may be various explanations for this finding. Although differences between populations may exist in terms of BMI and metabolic syndrome (to which

adiponectin is strongly related) [34], it is less likely that these differences fully explain the discrepancies as the leptin association is highly consistent and is also strongly associated to BMI and the metabolic syndrome [35]. Additionally we attempted to elucidate why ERF showed a very different association from the other populations by additionally adjusting for metabolic syndrome, but this did not change the results. When analyzing the adiponectin/leptin ratio in association with RTL in ERF, no significant correlation was found ($r = 0.005$, $p = 0.849$). Therefore, it is most likely that the association in ERF was a chance finding.

It is currently unclear whether high adiponectin levels are part of a protective effect due to an ongoing inflammation or whether individuals with low adiponectin levels are prone to inflammation. Another possibility would be that changes in adiponectin levels might be an epiphenomenon. Several prospective studies reported an association of high rather than low adiponectin levels with outcomes such as (cardiovascular) mortality [36–40], progression of chronic kidney disease [41–43], dementia [44] and type 2 diabetes [45]. This positive association was mainly observed in patients suffering already at baseline from a chronic disease. Other studies reported low adiponectin levels to be associated with several diseases such as insulin resistance [46–48], type 2 diabetes [49–51], atherosclerosis or cardiovascular disease [52]. It has been proposed that reverse epidemiology [14] or adiponectin resistance [53, 54] may be an explanation.

The association of leptin with RTL and lack of association of adiponectin with RTL leaves us with some interesting speculations considering the significance of our findings to the field. As leptin has been consistently associated with cardiovascular traits [1, 33] and here we also associated leptin with RTL, one of the implications to speculate on is to use RTL as a biomarker for coronary heart disease risk. The lack of association between RTL and adiponectin in individuals who do not suffer from coronary heart disease on the other hand suggests that a causal relationship between adiponectin and coronary heart disease is less likely and supports the view of reverse epidemiology [14].

A limitation of our study is that we do not have detailed measurements of adiposity, except BMI. There are many better adiposity markers in existence, including lean mass, waist circumference, waist to hip ratio and height to weight ratio, however these measurements were not available in all cohorts. Adjusting for these markers did not change the results in the ERF population. In the meta-analysis we only adjusted for BMI, which did not change our results for either adiponectin or leptin. However, we cannot completely exclude that adjusting for some other adiposity measurement might have changed the results. We have not considered other life-style based factors reported to be associated with RTL as many replicate poorly [55]. Observed differences in telomere length measurements across cohorts are mainly related to age differences between the various populations.

A major strength of the present study is its large sample size. This large number of samples requires telomere measurement to be done by a qPCR method instead of Southern Blot techniques. This approach for assessing RTL has some important advantages, including the high-throughput capacity and the requirement of only low amounts of DNA. However, we are also aware of the potential limitations of this method which are e.g. the type of the reference sample [56]. However, if performed in a highly standardized way, telomere qPCR is a valuable method for large epidemiological studies without consuming large amounts of DNA. The alternatively used method of telomere length measurement is the telomere restriction fragment (TRF) analysis (Southern Blot) where the absolute telomere length (kb) is estimated directly from a telomeric smear signal from blot pictures. However, this approach consumes a considerable amount of DNA, the required reagents are costly, and the methodology itself is cumbersome and labor-intensive. The results of the Southern Blot method can be influenced by several factors such as restriction enzymes, hybridization targets, hybridization probes and hybridization conditions, gel calibration, background subtraction, as well as the calculation formula and the analysis window [56]. Despite these limitations, to compare results of different studies, accurately measured absolute telomere length might be a more adequate and concrete method as a relative approach by qPCR. However, since the measurements in our meta-analyses were performed to a large extent in one laboratory and difference between methods were considered by using a Z-transformation of the data, we consider the qPCR method with the large number of samples as a major advantage.

On the flip-side of the equation, leptin and adiponectin measurements were not performed centrally and different assays were used for the measurements. Since many different assays have been used to measure adiponectin and leptin, which are based on different antibodies that undoubtedly detect different epitopes, we cannot exclude that some assays would give different results in the same population. Especially for adiponectin, this may be a problem, since it circulates in different oligomeric forms (low, medium and high molecular weight forms) [57–59]. Different assays will differ in the oligomeric forms that they measure. The different oligomeric forms differ in function and many of the beneficial effects have been attributed to the High molecular weight form [60, 61]. The fact that many assays have been used may have masked some associations.

Another advantage of our study is the population-based and multicenter design which allows the evaluation of consistent findings over populations. Though the meta-analysis also includes studies with relatedness (ERF, LLS, TwinsUK, Finnish twins), the relatedness of subjects within families was taken into account statistically in order to derive correct SEs and *p* values. The impact of modern living environment is undisputable. Nutrition, activity as well as the exposure to a wide range of man-made chemicals plays an important role and may have an effect on insulin action, metabolic rate and other physiological processes [62, 63]. Nevertheless, we observed an association of RTL and leptin in population-based studies of unrelated individuals as well as studies with related individuals sampled from the population without ascertainment for any disease.

In summary, our study is by far the largest addressing the relation between RTL and adipocytokines. We found consistent and significant evidence for an inverse correlation between RTL and leptin, which was not caused by increased BMI or CRP. However, we could not find a consistent association between RTL and adiponectin. This study has elucidated the association between adipocytokines and RTL in both sexes.

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References

1. Tilg H, Moschen AR. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. Nat Rev Immunol. 2006;6(10):772–83.
2. Conde J, et al. At the crossroad between immunity and metabolism: focus on leptin. Expert Rev Clin Immunol. 2010;6(5):801–8.
3. Hui X, et al. Adiponectin and cardiovascular health: an update. Br J Pharmacol. 2012;165(3):574–90.
4. Enriori PJ, et al. Leptin resistance and obesity. Obesity (Silver Spring). 2006;14(Suppl 5):254S–8S.
5. Blackburn EH, Gall JG. A tandemly repeated sequence at the termini of the extrachromosomal ribosomal RNA genes in Tetrahymena. J Mol Biol. 1978;120(1):33–53.
6. Blackburn EH, Greider CW, Szostak JW. Telomeres and telomerase: the path from maize, tetrahymena and yeast to human cancer and aging. Nat Med. 2006;12(10):1133–8.
7. Halvorsen TL, et al. Accelerated telomere shortening and senescence in human pancreatic islet cells stimulated to divide in vitro. J Endocrinol. 2000;166(1):103–9.
8. Kurz DJ, et al. Chronic oxidative stress compromises telomere integrity and accelerates the onset of senescence in human endothelial cells. J Cell Sci. 2004;117(Pt 11):2417–26.

9. von Zglinicki T. Oxidative stress shortens telomeres. Trends Biochem Sci. 2002;27(7):339–44.
10. von Zglinicki T, et al. Mild hyperoxia shortens telomeres and inhibits proliferation of fibroblasts: a model for senescence? Exp Cell Res. 1995;220(1):186–93.
11. Brouilette S, et al. White cell telomere length and risk of premature myocardial infarction. Arterioscler Thromb Vasc Biol. 2003;23(5):842–6.
12. Brouilette SW, et al. Telomere length, risk of coronary heart disease, and statin treatment in the West of Scotland Primary Prevention Study: a nested case-control study. Lancet. 2007;369(9556):107–14.
13. Fitzpatrick AL, et al. Leukocyte telomere length and cardiovascular disease in the cardiovascular health study. Am J Epidemiol. 2007;165(1):14–21.
14. Kalantar-Zadeh K, et al. Epidemiology of dialysis patients and heart failure patients. Semin Nephrol. 2006;26(2):118–33.
15. Aviv A, et al. Menopause modifies the association of leukocyte telomere length with insulin resistance and inflammation. J Clin Endocrinol Metab. 2006;91(2):635–40.
16. Diaz VA, et al. Telomere length and adiposity in a racially diverse sample. Int J Obes (Lond). 2010;34(2):261–5.
17. Njajou OT, et al. Shorter telomeres are associated with obesity and weight gain in the elderly. Int J Obes (Lond). 2011;36:1176.
18. Njajou OT, et al. Shorter telomeres are associated with obesity and weight gain in the elderly. Int J Obes (Lond). 2012;36(9):1176–9.
19. Valdes AM, et al. Obesity, cigarette smoking, and telomere length in women. Lancet. 2005;366(9486):662–4.
20. Zhu H, et al. Leukocyte telomere length in healthy Caucasian and African-American adolescents: relationships with race, sex, adiposity, adipokines, and physical activity. J Pediatr. 2011;158(2):215–20.
21. Al-Attas OS, et al. Adiposity and insulin resistance correlate with telomere length in middle-aged Arabs: the influence of circulating adiponectin. Eur J Endocrinol. 2010;163(4):601–7.
22. Aulchenko YS, et al. Linkage disequilibrium in young genetically isolated Dutch population. Eur J Hum Genet. 2004;12(7):527–34.
23. Pardo LM, et al. The effect of genetic drift in a young genetically isolated population. Ann Hum Genet. 2005;69(Pt 3):288–95.
24. Wichmann HE, et al. KORA-gen–resource for population genetics, controls and a broad spectrum of disease phenotypes. Gesundheitswesen. 2005;67(Suppl 1):S26–30.
25. Schoenmaker M, et al. Evidence of genetic enrichment for exceptional survival using a family approach: the Leiden Longevity Study. Eur J Hum Genet. 2006;14(1):79–84.
26. Beekman M, et al. Chromosome 4q25, microsomal transfer protein gene, and human longevity: novel data and a meta-analysis of association studies. J Gerontol A Biol Sci Med Sci. 2006;61(4):355–62.
27. Moayyeri A et al. Cohort profile: TwinsUK and healthy ageing twin study. Int J Epidemiol. 2012.
28. Heid IM, et al. Genetic architecture of the APM1 gene and its influence on adiponectin plasma levels and parameters of the metabolic syndrome in 1,727 healthy Caucasians. Diabetes. 2006;55(2):375–84.
29. Kaprio J. Twin studies in Finland 2006. Twin Res Hum Genet. 2006;9(6):772–7.
30. Pietilainen KH, et al. Growth patterns in young adult monozygotic twin pairs discordant and concordant for obesity. Twin Res. 2004;7(5):421–9.
31. Cawthon RM. Telomere measurement by quantitative PCR. Nucleic Acids Res. 2002;30(10):e47.
32. R Development Core Team. R: A language and environment for statistical computing. Vienna, Austria: R foundation for Statistical Computing; 2010.
33. Knudson JD, et al. Leptin and mechanisms of endothelial dysfunction and cardiovascular disease. Curr Hypertens Rep. 2008;10(6):434–9.
34. Okamoto Y, et al. Adiponectin: a key adipocytokine in metabolic syndrome. Clin Sci (Lond). 2006;110(3):267–78.
35. Considine RV, et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med. 1996;334(5):292–5.
36. Kistorp C, et al. Plasma adiponectin, body mass index, and mortality in patients with chronic heart failure. Circulation. 2005;112(12):1756–62.
37. Kizer JR, et al. Adiponectin and risk of coronary heart disease in older men and women. J Clin Endocrinol Metab. 2008;93(9):3357–64.
38. Maiolino G, et al. Plasma adiponectin for prediction of cardiovascular events and mortality in high-risk patients. J Clin Endocrinol Metab. 2008;93(9):3333–40.
39. Menon V, et al. Adiponectin and mortality in patients with chronic kidney disease. J Am Soc Nephrol. 2006;17(9):2599–606.
40. Pilz S, et al. Adiponectin and mortality in patients undergoing coronary angiography. J Clin Endocrinol Metab. 2006;91(11):4277–86.
41. Jorsal A, et al. Serum adiponectin predicts all-cause mortality and end stage renal disease in patients with type I diabetes and diabetic nephropathy. Kidney Int. 2008;74(5):649–54.
42. Kollerits B, et al. Gender-specific association of adiponectin as a predictor of progression of chronic kidney disease: the Mild to Moderate Kidney Disease Study. Kidney Int. 2007;71(12):1279–86.
43. Saraheimo M, et al. Serum adiponectin and progression of diabetic nephropathy in patients with type 1 diabetes. Diabetes Care. 2008;31(6):1165–9.
44. van Himbergen TM, et al. Biomarkers for insulin resistance and inflammation and the risk for all-cause dementia and alzheimer disease: results from the framingham heart study. Arch Neurol. 2012;69:594.
45. Li S, et al. Adiponectin levels and risk of type 2 diabetes: a systematic review and meta-analysis. JAMA. 2009;302(2):179–88.
46. Costacou T, et al. The prospective association between adiponectin and coronary artery disease among individuals with type 1 diabetes. The Pittsburgh epidemiology of diabetes complications study. Diabetologia. 2005;48(1):41–8.
47. Pischon T, et al. Plasma adiponectin levels and risk of myocardial infarction in men. JAMA. 2004;291(14):1730–7.
48. Schulze MB, et al. Adiponectin and future coronary heart disease events among men with type 2 diabetes. Diabetes. 2005;54(2):534–9.
49. Arita Y, et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun. 1999;257(1):79–83.
50. Hu E, Liang P, Spiegelman BM. AdipoQ is a novel adipose-specific gene dysregulated in obesity. J Biol Chem. 1996;271(18):10697–703.
51. Maeda N, et al. Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. Nat Med. 2002;8(7):731–7.
52. Han SH, et al. Antiatherosclerotic and anti-insulin resistance effects of adiponectin: basic and clinical studies. Prog Cardiovasc Dis. 2009;52(2):126–40.
53. Furuhashi M, et al. Possible impairment of transcardiac utilization of adiponectin in patients with type 2 diabetes. Diabetes Care. 2004;27(9):2217–21.
54. Kadowaki T, Yamauchi T. Adiponectin and adiponectin receptors. Endocr Rev. 2005;26(3):439–51.

55. Nordfjall K, et al. Telomere length is associated with obesity parameters but with a gender difference. Obesity (Silver Spring). 2008;16(12):2682–9.
56. Horn T, Robertson BC, Gemmell NJ. The use of telomere length in ecology and evolutionary biology. Heredity (Edinb). 2010;105(6):497–506.
57. Tsao TS, et al. Role of disulfide bonds in Acrp30/adiponectin structure and signaling specificity. Different oligomers activate different signal transduction pathways. J Biol Chem. 2003;278(50):
50810–7.
58. Waki H, et al. Impaired multimerization of human adiponectin mutants associated with diabetes. Molecular structure and multimer formation of adiponectin. J Biol Chem. 2003;278(41):
40352–63.
59. Wang Y, et al. Proteomic and functional characterization of endogenous adiponectin purified from fetal bovine serum. Proteomics. 2004;4(12):3933–42.
60. Hara K, et al. Measurement of the high-molecular weight form of adiponectin in plasma is useful for the prediction of insulin resistance and metabolic syndrome. Diabetes Care. 2006;29(6):1357–62.
61. Komura N, et al. Clinical significance of high-molecular weight form of adiponectin in male patients with coronary artery disease. Circ J. 2008;72(1):23–8.
62. Koch HM, Calafat AM. Human body burdens of chemicals used in plastic manufacture. Philos Trans R Soc Lond B Biol Sci. 2009;364(1526):2063–78.
63. Newbold RR. Impact of environmental endocrine disrupting chemicals on the development of obesity. Hormones (Athens). 2010;9(3):206–17.