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**Development of fatness from adolescence into adulthood
is adversely associated with leptin but not adiponectin
levels in adulthood: a 23-year follow-up study**

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Submitted

ABSTRACT

Background: Increased body fatness in youngsters is a risk factor for cardiovascular disease in adulthood. Hyperleptinaemia and hypoadiponectaemia have been proposed as molecular mechanisms that link adiposity to increased cardiovascular risk. However, the development of body fatness through adolescence and early adulthood in relation to adverse levels of these adipokines later in life is not clear.

Objective: To investigate, in healthy young adults, the longitudinal development of fatness parameters from the age of 13 to 36 years for groups with favourable and unfavourable levels of adipokines in adulthood.

Methods: We examined longitudinal data on fatness parameters (8 repeated measures between the ages of 13 and 36 years) in 377 subjects in whom levels of leptin and adiponectin were measured at age 36. The development of BMI, sum of skinfolds and skinfold ratio throughout the longitudinal period was compared between subjects across sex-specific tertiles of leptin and adiponectin. Data were analyzed with generalized estimating equations.

Results: Subjects in the highest tertile of leptin had higher mean values and a steeper increase in BMI and sum of skinfolds at all time points compared with subjects in the lowest tertile. These differences were already statistically significant during adolescence, and increased in magnitude from the age of 21 onward. In contrast, unfavourable levels of adiponectin at age 36 were not preceded by higher fatness during adolescence.

Conclusion: Higher levels of total fatness and BMI during adolescence and early adulthood are adversely associated with levels of leptin, but not of adiponectin, at age 36.

INTRODUCTION

Body fatness, in particular a central pattern of fat distribution, is independently associated with increased cardiovascular risk [1,2] and mortality [3]. The prevalence of (abdominal) obesity is increasing, especially in young individuals [4,5]. Longitudinal studies of children followed into young adulthood suggest that overweight children tend to become overweight adults, particularly if obesity (severe overweight) is present in adolescence [6-8]. Besides this, most of the available evidence, but not all [9], suggest that overweight in childhood/adolescence is associated with adverse health consequences in adulthood [10-12]. Also, there is mounting evidence that the association between elevated body mass index (BMI) or abdominal fat mass on the one hand, and cardiovascular risk on the other, is more or less linear. This implies that these associations are not confined to individuals with overweight or obesity, but are seen across the entire range of levels of body mass/fatness [13-17].

Until recently, adipose tissue was exclusively considered as a body energy store. However, it has become increasingly clear that adipose tissue is a source of circulating adipokines, such as leptin and adiponectin [18]. Specifically, hyperleptinaemia and hypoadiponectinaemia have been proposed as molecular mechanisms that link adiposity, predominantly central fatness, to increased cardiometabolic risk in both adults and children [19-24].

Although it is evident from the literature that an elevated BMI in adolescence constitutes a substantial risk factor for obesity-related disorders in midlife [13], it has not yet been completely delineated how the time courses of different fat parameters are with respect to favourable or unfavourable adipokine profiles across the entire range from normal weight to obesity.

In view of these considerations, we investigated these issues in a cohort of healthy young individuals who have been followed up from the age of 13 to the age of 36 years and in whom levels of adipokines (ie, adiponectin, leptin) were assessed at age 36. Specifically, we analyzed to what extent the longitudinal development of fatness parameters from the age of 13 to the age of 36 years were associated with favourable or unfavourable levels of adipokines at the age of 36 years, and also, whether we could detect a certain critical age/period in which the groups with favourable and

unfavourable levels of adipokines at the age of 36 began to differ substantially with respect to their fitness parameters over time.

SUBJECTS AND METHODS

Subjects and study design

Data were derived from the Amsterdam Growth and Health Longitudinal Study, an observational, longitudinal study that started in 1976 with a group of 698 boys and girls (details described elsewhere) [25]. Briefly, its initial goal was to study the natural development of the growth, health and lifestyle of adolescents and to investigate longitudinal relationships between biological and lifestyle variables. The mean age of the subjects at the start of the study was 13.1 ± 0.8 [mean \pm standard deviation (SD)] years. Since then, extensive follow-up measurements have been obtained 2 to 8 times (at the ages of 13, 14, 15, 16, 21, 27, 32, and 36 years) during a 24-year follow-up period and the cohort is still under investigation. At each follow-up measurement, anthropometric (body height, weight and skinfolds), biological (blood pressure, serum lipoprotein levels and physical fitness), lifestyle (nutritional habits, smoking behavior and daily physical activity) and psychological variables were assessed [25]. In the year 2000 (8th measurement round), when subjects' mean age was 36.6 (± 0.6) years, blood samples were taken in order to determine levels of adipokines (leptin and adiponectin) for the first time in 377 individuals. The present study reports on these 377 subjects (200 women) in whom complete measurements of adipokines were available. The study was approved by the local ethics committee of the VU University Medical Center, and all participants gave their written informed consent for each measurement round, which was provided by the parents when individuals were aged 13-16 years.

Adiponectin and leptin

Serum leptin and plasma adiponectin were measured in blood samples collected in the 2000 measurement rounds, when the subjects were 36 years old (stored at -80°C). Leptin was determined in serum samples with a 2-plex multi-array (MesoScale

Discover -MSD, Gaithersburg, MD, USA) as measured in a 96-well MULTI-SPOT plate. All reagents were provided with the MSD kit. Each 96-well plate has 2 spots per well with each spot pre-coated with anti-leptin antibodies. Samples, standards and controls were added at 25 μ l per well and the plate was incubated for 2h at room temperature. At the end of the incubation, the wells were washed and the electrochemiluminiscent-labeled detection antibody was added at 25 μ l per well and incubated for 1h at room temperature. For the detection, 150 μ l of the MSD Read Buffer was added to each well and the MSD plates were measured on the MSD Sector Imager 2004 plate reader as electro-chemiluminescence signal (light) detected by photodetectors and analyzed by using the Discovery Workbench 3.0 software (MSD). A logistic fit curve was generated for each marker using the standards and the concentration of each sample was calculated. Plasma total adiponectin was determined, as previously described, by a in-house time-resolved immunofluorometric assay based on two monoclonal antibodies and recombinant human adiponectin (R&D Systems, Abingdon, UK) [23]. The adiponectin molecule is known to form a wide range of polymers, of which the predominant polymers include trimers, hexamers and highly congregated multimers. Previous experiments have demonstrated that both monoclonal antibodies used are able to detect several adiponectin polymers in serum, including the three major molecular forms. All standards and unknown samples were analyzed in duplicate, with the exception of non-specific binding, which were analyzed in quadruplicate. The intra- and inter-assay coefficients of variation for both leptin and adiponectin assessments were <5% and <10%, respectively.

Anthropometry and body composition

From the age of 13 to 36 years, we repeatedly measured standing height, body weight, and the thickness of the biceps, triceps, subscapular, and suprailiac skinfolds [25-27]. We calculated, three indicators of total body fatness: 1) the body mass index (BMI, calculated as weight in kilograms divided by the square of height in meters), 2) the sum of the thickness of the 4 skinfolds, and 3) the ratio of the subscapular plus the suprailiac skinfold and the sum of all four skinfolds, which was used as an estimate of subcutaneous trunk fat [26,27].

Covariates

Lifestyle variables were assessed throughout the 24-year study period, (ie, alcohol consumption, smoking behaviour, physical activity and dietary intake) and biological risk factors (ie, sitting blood pressure and blood lipids) as described in detail elsewhere [25,26,28,29].

Statistical Analysis

We used generalized estimating equations (GEEs) to investigate the time course of BMI, fatness (sum of skinfolds) and subcutaneous trunk fat (skinfold ratio) in the subjects who were identified as with *favourable* versus *unfavourable* adipokine levels (i.e., leptin and adiponectin) at the age of 36 years. In order to do so, we created sex-specific tertiles of each of the adipokines. In the analyses the ‘most favourable’ tertile (i.e. low leptin, high adiponectin) was compared with the ‘least favourable’ tertile (vice versa) for both leptin and adiponectin.

In all GEE analyses, time was treated as a categorical variable and an exchangeable correlation structure was assumed. GEE analyses adjust for the correlation between repeated observations taken in the same subject and has the advantage of handling longitudinal data on subjects with varying number and unequally spaced observations [30].

All analyses were performed for men and women together because no significant interaction by gender was found. All statistical analyses were performed using the Statistical Package for Social Sciences for Windows (SPSS version 17.0, SPSS Inc, Chicago, IL, USA).

RESULTS

Table 1 shows the general characteristics of the study population throughout the longitudinal period. Both BMI, sum of skinfolds and skinfold ratio increased from age 13 to age 36. Table 2 shows the median (inter-quartile range) of leptin and adiponectin across tertiles.

Table 1. Characteristics of the study population throughout the 24-year longitudinal period

Variables	Calendar age							
	13	14	15	16	21	27	32	36
BMI, kg/m ²	17.4±1.8	18.2±1.9	18.8±2.0	19.6±2.0	21.4±2.2	22.2±2.3	23.3±2.9	24.1±3.2
Sum of 4 skinfolds, mm*	31.7±12.1	33.5±12.8	36.2±15.3	37.7±15.6	45.0±17.4	42.0±16.1	47.7±19.2	52.2±19.1
Skinfold ratio †	0.49±0.06	0.51±0.06	0.53±0.06	0.54±0.06	0.58±0.08	0.56±0.08	0.56±0.09	0.57±0.10
Other biological risk factors								
Systolic blood pressure, mm Hg‡	126.6±9.3	123.4±9.2	124.9±10.0	125.7±10.7	128.6±11.1	129.1±11.9	129.7±12.4	131.2±14.3
Diastolic blood pressure, mm Hg‡	75.5±7.8	75.9±7.5	72.4±8.1	74.5±8.1	78.6±8.1	80.9±8.5	84.6±8.8	85.4±10.6
Pulse pressure, mm Hg‡	49.2±9.9	47.5±10.1	52.6±11.9	51.2±11.4	50.1±10.5	48.3±10.7	45.1±9.9	45.9±9.8
Total cholesterol, mmol/L	4.47±0.73	4.36±0.71	4.31±0.73	4.25±0.78	4.72±0.81	5.09±0.96	4.94±0.85	5.00±0.93
HDL cholesterol, mmol/L	1.45±0.29	1.42±0.26	1.30±0.25	1.36±0.27	1.30±0.29	1.43±0.38	1.42±0.37	1.41±0.37
Total:HDL cholesterol ratio	3.2±0.7	3.2±0.7	3.4±0.8	3.2±0.7	3.8±0.9	3.8±1.0	3.7±1.2	3.8±1.3
Lifestyle risk factors								
Alcohol consumption, %	11.3	14.7	30.5	46.7	68.9	72.4	77.9	81.4
Smoking, %	1.6	10.8	13.3	17.6	30.6	27.0	18.3	23.2
Physical activity, 10 ³ METs/wk	4.40±1.83	3.97±1.62	3.72±1.61	3.51±1.55	3.34±2.10	3.04±2.02	3.41±2.29	4.90±3.27
Total energy intake, 1000 kcal/d	2.46±0.55	2.49±0.59	2.58±0.70	2.55±0.69	2.61±0.74	2.48±0.66	2.60±0.71	2.61±0.69

Data are means ±SD or percentages;

*Data show the sum of the thickness of the following skinfolds: triceps, biceps, subscapular and suprailliac;

†Ratio calculated as (subscapular + suprailliac)/sum of 4 skinfolds;

‡ Measurements were performed with a sphygmomanometer on the right arm with subjects in the sitting position after ≥5 minutes of rest.

Table 2. Levels of leptin, adiponectin and LAR at the age of 36 across tertiles of each adipokine per sexe

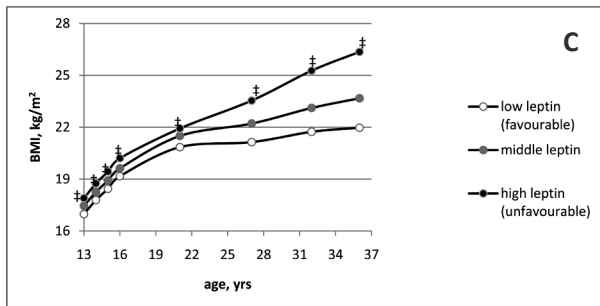
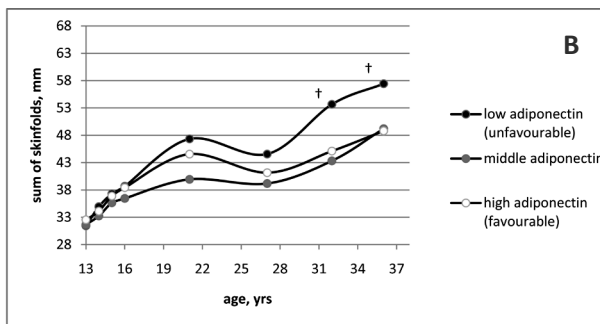
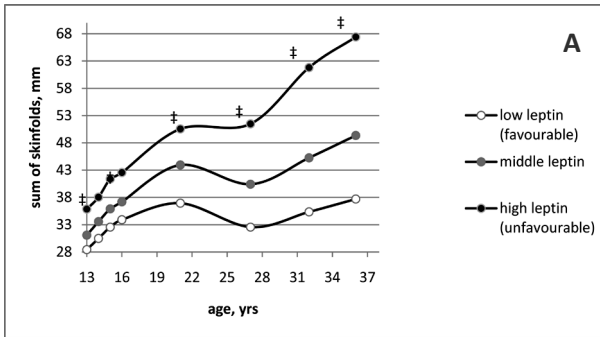
Adipokine level	T1	T2	T3
Men			
Leptin, µg/L	1.36 [1.05-1.81]	3.06 [2.56-3.78]	6.81 [5.38-9.48]
Adiponectin, mg/L	4.94 [4.30-5.42]	7.20 [6.33-7.87]	9.81 [8.83-11.16]
Women			
Leptin, µg/L	5.28 [4.04-6.08]	10.07 [8.48-11.55]	21.19 [17.49-35.25]
Adiponectin, mg/L	7.19 [6.16-8.73]	10.58 [10.13-11.13]	14.40 [12.51-16.09]

Data are medians [inter-quartile range].

T1 indicates lowest tertile; T2, middle tertile; and T3, highest tertile of each adipokine level.

Time course of sum of skinfolds, BMI and skinfold ratio

Figure A to F show the development of sum of skinfolds, BMI and the skinfold ratio for subjects with favourable versus non-favourable levels of adiponectin and leptin. As for differences in developmental patterns between tertiles of adipokine levels at age 36, the sum of skinfolds showed larger differences for leptin (Figure A) as compared to adiponectin (Figure B). For BMI (Figure C and D), the same patterns were found as for sum of skinfolds, although the differences between the tertiles were less pronounced. For the skinfold ratio (Figure E and F), a similar pattern over time was observed, albeit with smaller absolute differences and higher p-values. As for the time course of the various curves, a fairly consistent observation was that the developmental patterns of fatness parameters showed earlier separation for leptin (i.e. significant differences already apparent at first measurement at age 13) than for adiponectin (i.e. consistent separation starting at age 32, Figure B and D).



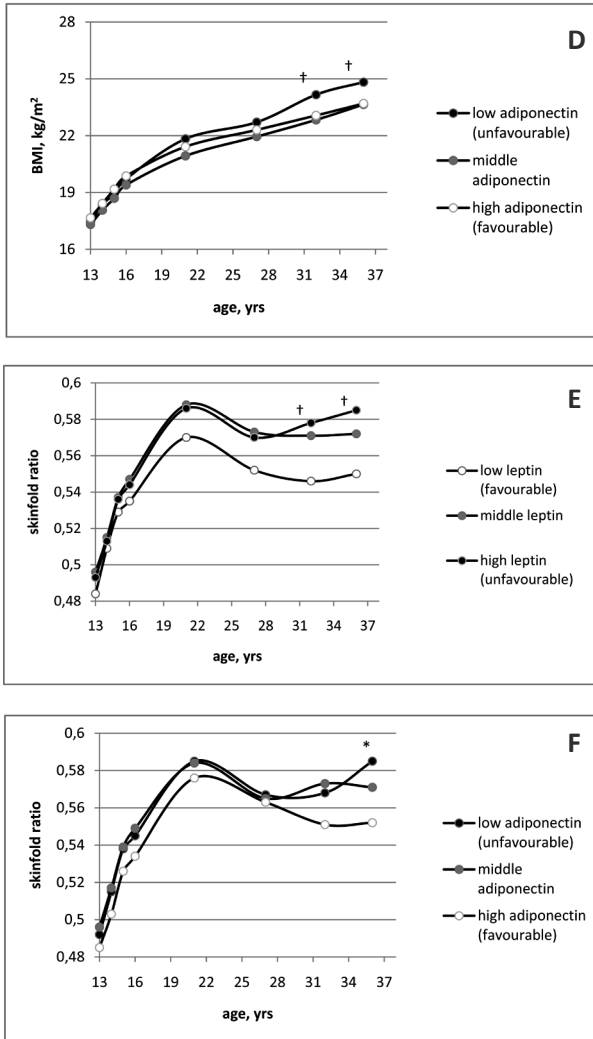


Figure. Longitudinal development of sum of skinfolds (Figure A and B), BMI (Figure C and D) and the skinfold ratio (Figure E and F) for subjects with the most favourable versus non-favourable levels of adiponectin and leptin. The asterisk indicates $P < 0.05$; dagger, $P < 0.01$; and double dagger, $P < 0.001$.

DISCUSSION

In the present study we compared the longitudinal development of fatness parameters from the age of 13 to 36 years between groups with favourable and unfavourable levels of adipokines at age 36. In addition, we investigated whether we could detect a particular critical age at which the patterns began to differ substantially between these groups.

The main findings of this study are two-fold. First, the largest differences in developmental pattern of fatness parameters were found between subjects with favourable versus unfavourable levels in leptin at the age of 36. Second, small but statistically significant differences between these leptin groups in fatness parameters were already observed at the age of 13 and these differences became evidently larger especially from the age of 21 onward. The differences in the developmental pattern of fatness parameters between favourable and unfavourable levels of adiponectin were significant only at the age of 32 and 36 years.

In previous cross-sectional studies, adverse adiponectin levels were noted in obese children as compared to normal-weight children [31,32]. Further, it has been suggested that adiponectin and leptin play a key role in the pathogenesis and complications of obesity in both children and adults [33]. No previous studies, however, have addressed the development of fatness over a prolonged period of time in relation to levels of adipokines in adulthood. We are aware of only one single cohort study in 490 subjects with a limited 2-year follow-up period examining the effect of varying degrees of fatness in childhood on the level of adiponectin. They found that adverse (ie, low) levels of adiponectin were already present in obese youngsters (4-20 years of age), and that these levels worsened with the degree of obesity [5].

In the present study, larger and earlier differences in developmental pattern of estimates of total fat were found between subjects with favourable versus unfavourable levels in leptin as compared with adiponectin. Unfortunately, we had no data on adipokines throughout the course of life of our study population, but only at age 36 onward. As a result, we were not able to investigate if adverse levels were already present in adolescence. Leptin provides signals about nutritional status and fat mass to neural centres that regulate appetite and energy metabolism. Leptin

levels are usually higher in obese relative to lean child and adult populations [20,34]. Any weight loss due to a decrease in adipose mass corresponds with a decrease in circulating leptin concentrations [35-37]. This is in line with the theory of a leptin resistant state existing in most obese humans, whereby there is a defect within the leptin signalling cascade system [20,34,38]. Several experimental studies have shown that both leptin and adiponectin may, respectively, promote or prevent endothelial dysfunction, oxidative stress, platelet aggregation, and migration, hypertrophy and proliferation of smooth muscle cells [20,22,38,39], all of which may influence the risk of cardiometabolic disease. However, the role of adipokines in early life in the pathogenesis of cardiometabolic complications in adulthood is still a matter of debate. It was suggested that adiponectin in children could be a strong predictor for development of the metabolic syndrome, whereas the role for leptin seemed to be more important in states of energy deficiency [24]. Another review concluded that leptin is of interest as a key adipokine in childhood obesity and its cardiometabolic sequelae [34]. All together, it suggests that adiponectin and leptin may both play a role as a link between obesity and cardiovascular disease but through different pathways, as well as in childhood as in adulthood.

An important point of concern in epidemiological studies of total fat and the distribution of body fat in youth is the choice of the most appropriate fatness indicator. Total body fat can be predicted from the measurement of skinfolds at several sites, both in childhood and adolescence [40]. The use of body mass index (BMI) is more reproducible than (the sum of) skinfolds, but its correlation with total body fat is weaker, since it does not distinguish between fat mass and lean (non-fat) mass [41]. This is also reflected in our findings, as the largest differences in fatness patterns between subjects with favourable versus unfavourable adipokine levels were found in the sum of skinfolds, and not in BMI.

Body fat distribution is known to relate to future health in adults [16], but this has not yet been confirmed in children. We assessed body fat distribution by the ratio of skinfold thickness at trunk and extremity sites. A reason why we did not find large differences in body fat distribution (as compared to total fat) between subjects with favourable versus unfavourable leptin levels, could be that leptin levels are, so far, found to be more strongly associated with total (subcutaneous) fatness rather than

with the visceral fat depot [42]. Adiponectin on the other hand seems to be more sensitive than leptin to fat distribution [42,43], although we could not confirm this in the present study. Another potential reason for the lack of large differences in the pattern of fat distribution is that the potential of measurement error of skinfolds thickness is compounded in skinfold ratios, which weakens their association with any outcome measure [44]. Other indicators for the assessment of body fat distribution include dual x-ray absorptiometry (DXA), the use of waist circumference (WC) and the waist-to-hip ratio (WHR). The use of WHR, however, has not yet been validated in adolescents [45].

There are some limitations to our study. First, we have not measured adiponectin sub-forms, and the high-molecular weight (HMW) complex has been suggested to reflect metabolic abnormalities associated with childhood obesity [46]. It is thus possible that associations between HMW adiponectin and fatness parameters are stronger than the ones reported here. Second, our findings were confined to subjects attending the follow-up in 2000 (at age 36). Levels of BMI, and sum of skinfolds in these subjects were slightly lower than from those who dropped out (data not shown), suggesting that the associations found between adipokines and fatness parameters could possibly be an underestimation of the real associations. Third, the measurement of indicators of total body fat and a central pattern of body fat based on sum of skinfolds and skinfold ratio, respectively, are susceptible to measurement error, particularly if the subject has thick skinfolds [44]. However, it was shown in this same cohort, after comparison with DXA, that the sum of skinfolds is a reliable alternative for the measurement of total body fat mass across the whole range of values at the age of 36 [47]. This suggests that possible measurement error would be non-differential and thus result in a potential underestimation of the real associations.

In conclusion, our findings show that higher and increasing levels of total fatness and BMI, starting at early adolescence and throughout the course of life, are adversely associated with levels of leptin, but to a more modest degree with levels of adiponectin, at age 36. For leptin, the differences in fatness parameters became evidently larger especially after the age of 21 onward.

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