

Original article

Serum leptin and adiponectin levels and their association with allergic sensitization

Background: Obesity and respiratory allergies have increased in parallel in industrialized countries. We have recently shown an association between obesity and allergic sensitization whereby obesity diminished the protective effect of childhood farm contact.

Objective: To assess whether taking obesity into account allergic sensitization is associated with adipokine levels in blood and whether this effect is modified by childhood farm contact.

Methods: Serum samples of 231 adult participants (age 18–45 years) of the Lower Saxony Lung Study were analysed for leptin and adiponectin by ELISA. Subjects were elected to represent equal-sized groups with respect to obesity (< 30 vs ≥ 30 kg/m²), childhood farm contact, specific IgE to ubiquitous allergens and sex. Multiple logistic regression models were adjusted for potential confounders.

Results: Leptin levels were positively related to the prevalence of sensitization (highest vs lowest quartile odds ratio 6.7, 95% confidence interval 2.0–22.4). For adiponectin levels, a weak, not statistically significant inverse association with sensitization was shown (highest vs lowest quartile 0.4, 0.2–1.1). The association between leptin and sensitization appeared to be more pronounced in subjects with farm contact; however, the effect modification was not statistically significant.

Conclusion: These findings suggest that adipokines might be involved in the causal pathway between obesity and allergic sensitization.

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Key words: adipokines; adiponectin; hygiene hypothesis; inhalant allergens; leptin.

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Accepted for publication 19 February 2008

Over the past decades, the prevalence of both obesity and allergic sensitization has been increasing in parallel in industrialized countries. Recent epidemiological and experimental data suggest that obesity might be linked to allergic sensitization (1–9). This association might involve common factors with regard to genetic disposition and environmental influences (10).

One of the environmental factors associated with allergic sensitization is early childhood farm contact. Since the original ‘hygiene hypothesis’ has been set up by Strachan in 1989, a large number of studies have indicated that such childhood farm contact is linked to

a reduced risk of atopic diseases [for review see (11–13)]. Extending this view, we have recently shown that the protective effect of early childhood farm contact on allergic sensitization is diminished by adult obesity (14).

This observation seems of particular interest in face of the increasing prevalence of obesity. Still, however, the knowledge about pathophysiological links between obesity and sensitization is limited. Among the candidates are immunomodulatory effects of circulating compounds such as leptin that show elevated levels in obese subjects (15).

Leptin is an adipokine released by adipocytes. Laboratory studies have indicated that leptin is associated with a T helper cell (Th)1 response (7) but can also augment ovalbumin (OVA)-induced IgE production in sensitized mice (8), indicating its proinflammatory potential. In contrast, the expression of adiponectin, a further important adipokine, is diminished in obese subjects (16) and adiponectin can attenuate allergen responses in mice (17). As part of its anti-inflammatory action, adiponectin is capable of reducing tumor necrosis factor-alpha (TNF)- α -induced NF- κ B activation and lipopolysaccharide

Abbreviations: 95%CI, 95% confidence interval; ALEX-study, Allergy and Endotoxin Study; ANOVA, analysis of variance; BMI, body mass index; ECRHS, European Community Respiratory Health Survey; IgE, immunoglobulin E; IU, International Unit; LOESS, locally optimal estimating and smoothing scatter plots; LPS, lipopolysaccharide; NF- κ B, nuclear factor kappa B; OR, odds ratio; OVA, ovalbumin; SX1, mixture of ubiquitous specific allergens; RAST, radio-allergen-sorbent-test; Th, T helper cell; TNF- α , tumor necrosis factor-alpha.

(LPS)-induced TNF- α production (16). Such immunomodulatory factors are also thought to be involved in the protective effects of farm exposure (18).

These observations raise the question whether the associations between body weight, early childhood exposure and allergy (19) are related to blood levels of leptin and adiponectin, as two partially counteracting hormonal immunomodulatory compounds.

Based on these considerations, we assessed the relationship between serum leptin and adiponectin levels and sensitization against ubiquitous allergens in adult subjects with and without childhood farm contact. Taking obesity into account, the following hypotheses should be tested with this approach:

- Serum leptin levels are positively associated with allergic sensitization in adults.
- Serum adiponectin levels are inversely related to allergic sensitization in adults.
- These associations are modified by childhood farm contact.

Methods

Study subjects

For the present analysis, data of the clinical part of the Lower Saxony Lung Study, a cross-sectional study among rural adults (age 18–45 years) living in four towns in north-western Germany were used. Details of the study have been given previously (14, 20, 21). In short, 1820 subjects took part in the clinical examination (response 64%) between 2002 and 2004, fulfilled the inclusion criteria [main place of residence in the study area, physically able to participate, born in former west Germany, regular contact to farm animals before age 3 years or after age 18 years, not pregnant or breast feeding, body mass index (BMI) $> 18.5 \text{ kg/m}^2$] and had complete data.

For feasibility reasons and financial constraints, 240 subjects were chosen at random for the present analyses. Following our initial hypotheses, four equal sized groups were selected based on BMI and farm contact during the first 3 years of life (Fig. 1):

- Nonobese nonfarm subjects: BMI $< 30 \text{ kg/m}^2$ without farm contact during the first 3 years of life;

- Obese nonfarm subjects: BMI $\geq 30 \text{ kg/m}^2$ without farm contact during the first 3 years of life;
- Nonobese farm subjects: BMI $< 30 \text{ kg/m}^2$ and living on a farm or regular visits to animal houses during the first 3 years of life;
- Obese farm subjects: BMI $\geq 30 \text{ kg/m}^2$ and living on a farm or regular visits to animal houses during the first 3 years of life.

This way, we took into account that leptin and adiponectin levels depend on BMI. In addition, we were able to stratify our analyses for childhood farm contact as a potential effect modifying variable. To enrich the sample by subjects with allergic sensitization in each category, we additionally matched for sensitization [specific IgE to ubiquitous allergens (SX1) $\geq 0.35 \text{ IU/ml}$]. As it is known that serum adipokine levels also vary by sex (22–24), groups were additionally matched on sex.

In 2007, serum samples of the 240 randomly selected subjects were analysed for leptin and adiponectin. It turned out that in nine of the samples the volume was insufficient for analysis or they were coagulated and thus could not be used (Fig. 1).

The study was approved by the Medical Ethical Committee of the Ludwig-Maximilians-University Munich and the Lower Saxony Medical Board.

Questionnaire

The mail-in questionnaire used in the Lower Saxony Lung Study consisted of validated questions on respiratory health, demographic characteristics, potential confounders and farm contact. The items were mainly taken from the questionnaire of the European Community Respiratory Health Survey (ECRHS) (25) and the ALEX-study (26).

Farm contact during the first 3 years of life was defined present if subjects either answered 'yes' to the question 'Did you live on a farm during the first 3 years of your life?' or confirmed that they had visited animal houses regularly during the first 3 years of life (26).

Medical examination

Of the data obtained at the medical examination, BMI and specific IgE were used for the present analyses. Weight and height were measured and BMI calculated as weight divided by height squared (kg/m^2). Obesity was defined as BMI $\geq 30 \text{ kg/m}^2$.

Specific IgE against a panel of ubiquitous allergens (Timothy grass, rye, mugwort, birch, *Dermatophagoides pteronyssinus*, *Cladosporium herbarum*, cat and dog) was determined in serum samples (SX1, Pharmacia CAP System, Freiburg, Germany).

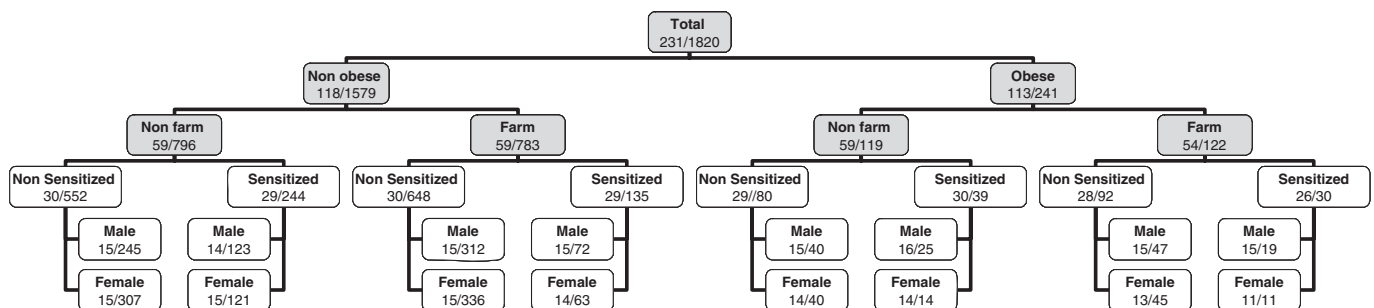


Figure 1. Stratified selection of the blood samples from the eligible study population. Wherever possible, a random sample of the subgroup was taken for the present study.

Subjects with specific IgE ≥ 0.35 kU/l (corresponding to RAST class 1 or higher) were regarded as sensitized (27).

Analysis of leptin and adiponectin serum levels

Quantikine® assays (Human Leptin Immunoassay, Human Adiponectin/Acrp30 Immunoassay; R&D Systems GmbH, Wiesbaden, Germany) were used to determine leptin and total adiponectin levels. These assays employed the quantitative sandwich enzyme immunoassay technique, using monoclonal antibodies specific for leptin or adiponectin that were precoated onto a microplate and applicable for serum samples.

Briefly, 10 μ l serum were added to 990 μ l diluent. From this, 100 μ l (adiponectin: 50 μ l) were transferred onto the plate, together with standards (leptin: 0–1000 pg/ml, adiponectin: 0–250 ng/ml). After washing, an enzyme-linked monoclonal antibody specific for leptin or adiponectin was added. Following a further wash, substrate solution was added. After stopping, color intensity was measured at 540 nm on a microplate reader (Powerwave; MWG-Biotech AG, Ebersberg, Germany) according to the manufacturer's instructions. All samples ranged above the minimum detectable serum concentrations (leptin: 0.3 μ g/l; adiponectin: 3.9 mg/l).

Statistical methods

For categorical variables, descriptive data are presented as absolute and relative frequencies, and chi-squared tests were used for comparisons. For continuous variables, median and range were calculated and values were compared by analysis of variance (Kruskal–Wallis ANOVA) for more group comparisons and by

Wilcoxon test for two group comparisons. A *P*-value of <0.05 was considered statistically significant.

Locally Optimal Estimating and Smoothing Scatter (LOESS) plots controlling for age and matching variables (sex; male, female), early childhood farm contact (no, yes) and BMI (<30 vs ≥ 30 kg/m²) were used to visualize the association between leptin, adiponectin and sensitization. A band width of 0.6 was used for these models.

In the next step, multiple logistic regression models were developed using quartiles of leptin (1st quartile ≤ 4.6 , 2nd ≤ 9.0 , 3rd ≤ 20.7 , 4th ≤ 85.9 μ g/l) and adiponectin (1st ≤ 5.6 , 2nd ≤ 8.0 , 3rd ≤ 11.7 , 4th ≤ 40.9 mg/l) as main predictors. These models were adjusted for the matching variables (sex, childhood farm contact and obesity). In bivariate models, age (five categories: 18–25, 26–30, 31–35, 36–40, 41–45 yrs), environmental tobacco smoke exposure (ETS; yes, no), smoking (never, ex, current), level of education (<12 vs ≥ 12 years of schooling) and number of siblings (≤ 2 vs >2) were associated with the levels of leptin or adiponectin and allergic sensitization. Therefore, these variables were also included in the multiple models as potential confounders.

Results

As shown in Table 1, obese and nonobese farm and nonfarm subjects did not show major differences with respect to age, number of siblings or smoking habits. However, the level of education was different ($P < 0.05$), with higher levels in nonobese nonfarm compared with farm subjects. Body mass index was similar in corre-

Table 1. Descriptive data of the study population stratified for childhood farm contact and obesity

	Nonobese farm	Obese farm	Nonobese nonfarm	Obese nonfarm
<i>N</i>	59	54	59	59
Descriptives				
Age (mean $\pm$ SD) (years)	35.5 $\pm$ 6.4	35.3 $\pm$ 5.9	32.5 $\pm$ 7.9	35.3 $\pm$ 7.7
Sex: female	29 (49.2%)	24 (44.4%)	30 (50.9%)	28 (47.5%)
Higher level of education*	14 (23.7%)	6 (11.1%)	19 (32.2%)	10 (17.0%)
Smoking				
Never	27 (45.8%)	29 (53.7%)	32 (54.2%)	27 (45.8%)
Ex	14 (23.7%)	18 (33.3%)	10 (17.0%)	10 (17.0%)
Current	18 (30.5%)	7 (13.0%)	17 (28.8%)	22 (37.3%)
ETS exposure	26 (44.1%)	26 (48.2%)	23 (39.0%)	36 (61.0%)
>2 siblings	33 (55.9%)	36 (66.7%)	25 (42.4%)	31 (52.5%)
Respiratory symptoms and sensitization				
Sensitization	29 (49.2%)	26 (48.2%)	29 (49.2%)	30 (50.9%)
Allergic rhinitis ever ($n_{\text{miss}} = 5$)	11 (19.0%)	10 (19.2%)	16 (27.6%)	14 (24.1%)
Symptomatic sensitization ($n_{\text{miss}} = 4$)	9 (15.5%)	10 (19.2%)	14 (23.7%)	14 (24.1%)
Wheeze last 12 months	6 (10.2%)	11 (20.4%)	6 (10.2%)	15 (26.4%)
Dr dx asthma ever ($n_{\text{miss}} = 4$)	2 (3.5%)	3 (5.7%)	5 (8.6%)	9 (15.5%)
Markers of obesity				
BMI [median (range)] (kg/m ²)**	23.9 (19.5; 29.4)	31.8 (30.1; 56.5)	23.9 (18.8; 29.9)	32.3 (30.1; 47.2)
Serum leptin [median (range)] (μ g/l)**	5.0 (0.3; 26.3)	15.0 (2.8; 85.9)	6.1 (0.5; 22.1)	20.7 (3.3; 75.9)
Serum adiponectin [median (range)] (mg/l)**	8.6 (2.3; 40.9)	7.4 (1.4; 23.1)	8.9 (1.6; 25.6)	6.6 (1.8; 17.1)

* $P_{\text{Chi-Square}} < 0.05$; ** $P_{\text{Kruskal-Wallis}} < 0.001$; n_{miss} , number of missings.

Sensitization: specific IgE to ubiquitous allergens ≥ 0.35 kU/l.

Obesity: BMI ≥ 30 kg/m².

Higher level of education: ≥ 12 years of schooling.

Symptomatic sensitization: specific IgE to ubiquitous allergens and symptoms of allergic rhinitis ever.

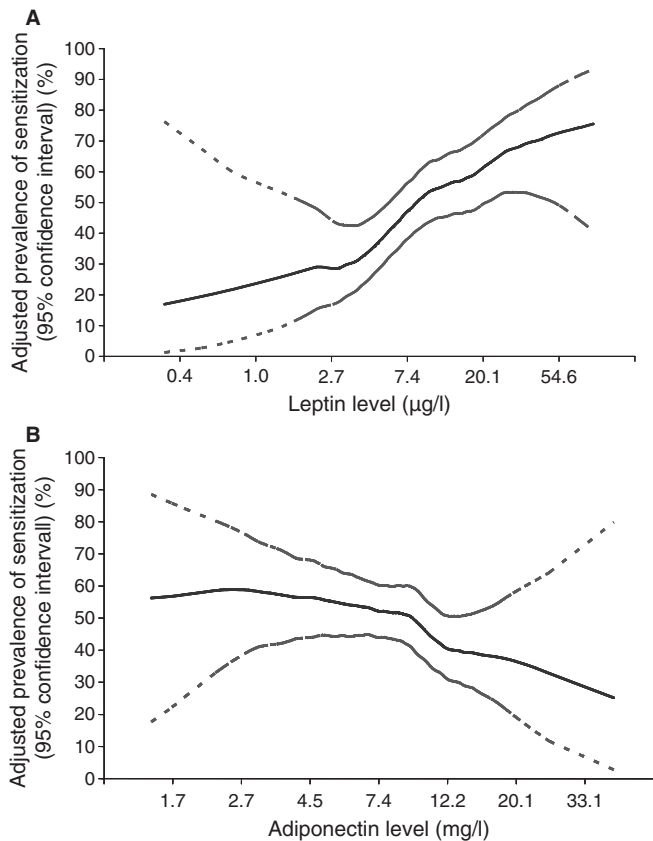


Figure 2. Smoothed plots with 95% confidence intervals of allergic sensitization in relation to leptin (A) and adiponectin (B). Adjusted for age, sex, farm contact and obesity.

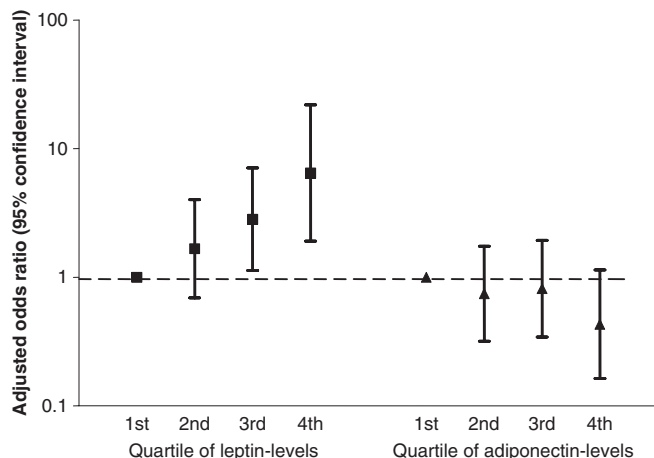


Figure 3. Odds ratio with 95% confidence interval for the association between serum leptin and adiponectin levels (in quartiles) and allergic sensitization. Adjusted for age (in categories), sex, smoking, ETS exposure, obesity and childhood farm contact.

sponding groups, i.e. in obese nonfarm compared with farm subjects and in nonobese nonfarm compared with farm subjects. As expected from our previous results (21),

the prevalence of respiratory symptoms was slightly but not significantly ($P > 0.05$) higher in nonfarm than in farm subjects despite an equal prevalence of allergic sensitization.

Adipokines and obesity

Obese subjects showed markedly higher serum leptin levels than subjects without obesity (Table 1). This was true for the total population, as well as for the subgroups with and without childhood farm contact ($P_{\text{Wilcoxon}} < 0.001$ each). Adiponectin levels were significantly lower in obese compared with nonobese subjects ($P_{\text{Wilcoxon}} < 0.001$) however, these differences were less pronounced. As for leptin, the differences in adiponectin between obese and nonobese subjects did not depend on childhood farm contact ($P_{\text{Wilcoxon}} < 0.01$ each).

Adipokines and sensitization

For the total population, leptin levels were statistically significantly elevated in sensitized (median 10.4; range 0.8–72.8 μg/l) compared with nonsensitized subjects (8.6; 0.3–85.9 μg/l; $P_{\text{Wilcoxon}} = 0.04$). In contrast, adiponectin levels did not differ significantly between sensitized (7.7; 1.64–24.9 mg/l) and nonsensitized subjects (8.6; 1.4–40.9 mg/l; $P_{\text{Wilcoxon}} = 0.09$). The same pattern was observed for the four subgroups (obese and nonobese subjects with and without childhood farm contact; data not shown).

Adjusted LOESS plots (Fig. 2) for the total study population indicated a linear positive association between serum leptin level and prevalence of allergic sensitization. The inverse association between adiponectin and sensitization was weaker and seemed to be confined to the highest quartile of adiponectin (> 11.7 mg/l).

In the next step, a multiple logistic regression model was developed including leptin and adiponectin levels in quartiles (Fig. 3). It confirmed the linear positive association between leptin and allergic sensitization. Odds ratios for sensitization were significantly increased for subjects with serum leptin levels in the 3rd (OR 2.8, 95% CI 1.1–7.0) and 4th quartile (OR 6.7, 95% CI 2.0–22.4) compared with the 1st quartile. In contrast, the association between adiponectin quartiles and allergic sensitization was not statistically significant.

To assess whether the association between serum leptin and allergic sensitization was modified by childhood farm contact, the logistic regression model was stratified for farm contact. To gain statistical power, adiponectin quartiles were not included in this model. The resulting association between leptin and sensitization was more pronounced in subjects with childhood farm contact than in those without such contact (Fig. 4). However, confidence intervals were wide and no statistically significant interaction between childhood farm contact and leptin

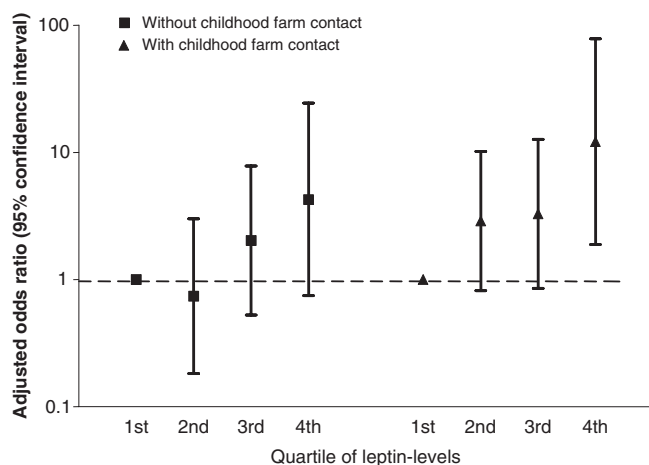


Figure 4. Odds ratio with 95% confidence interval for the association between serum leptin levels (in quartiles) and allergic sensitization stratified for childhood farm contact. Adjusted for age (in categories), sex, smoking, ETS exposure and obesity.

could be shown ($P_{\text{Interaction}} > 0.05$). The association remained basically the same when adiponectin was included but confidence intervals became wider (data not shown).

Discussion

To our knowledge, this is the first epidemiologic study in adults indicating a positive correlation between serum leptin levels and allergic sensitization to ubiquitous allergens. In addition, a weak, inverse association between serum adiponectin and allergic sensitization was demonstrated at high adiponectin levels. This finding provides further evidence that the association between obesity and asthma (9, 16, 28, 29) is not solely based on lung mechanical or genetic factors but might also be mediated by immunomodulatory, inflammatory mechanisms. Thus, among the factors by which obesity could increase the likelihood of asthma, adipokines and allergic sensitization also have to be taken into account.

As we were not able to analyse the whole study population, we increased the efficiency of our study by selecting blood samples based on subjects' obesity, childhood farm contact, allergic sensitization and sex. This way we increased the power of our analyses, although the selected samples cannot be assumed to be representative for the total population. Therefore, we cannot analyse the association between, for example, obesity and allergic sensitization. However, it enables us to analyse the association between adipokines and allergic sensitization independently of BMI.

Adipokine levels were determined carefully according to standard procedures by one experienced technician (G. D.-G.). Serum samples had been stored frozen at -20°C for 4 years until analysis and were never unfrozen.

Coagulated samples were excluded. Moreover, measurements were carried out in duplicate, showing excellent reproducibility. Therefore, differential misclassification of serum leptin or adiponectin levels is unlikely.

As we did not acquire data on nutritional habits, it was not possible to study the association between nutrition and adipokine levels or allergic sensitization. However, it seems unlikely that nutrition might have biased our findings.

Our results confirm earlier findings that obesity is linked to the blood levels of several adipokines (10, 15, 16). With respect to the association between leptin levels and allergic sensitization our epidemiological findings are in line with the only clinical study so far available on this issue (30). This recent study (30) was based on 26 patients with allergic rhinitis and 20 controls. In combination with experimental data in animals (8, 17), these findings suggest a link between adipokines and allergy.

Among the childhood factors that influence the risk for obesity in adulthood range birth weight, breast feeding, food habits, rate of maturation and activity (31–33). In the complex interplay of interacting and time-dependent risk factors it is currently not known whether childhood farm exposure modulates the risk of obesity in adulthood. Unfortunately, data on childhood BMI were not available for the study participants of the recent study. With respect to adult BMI, farm and nonfarm subjects ($n = 2469$) did not differ significantly (25.7 vs 25.3 kg/m^2 , respectively; $P = 0.67$).

We observed in our previous study that the protective effect of early childhood farm contact on allergic sensitization in adulthood was diminished in obese subjects (14). Among others, this raised the question whether there is an association between serum levels of adipokines and allergic sensitization in adults and whether this association is modified by childhood farm contact as a carry-over effect from childhood. The protective effect of farm contact seems to involve modulation of Th1-Th2 and T regulatory associated cytokine production (34). Such modulation of cytokine production also occurs in obesity (35) which makes an interaction between both possible. This would point towards a further aspect of (potentially epigenetic) carry-over effects from childhood and suggest a direct link between the respective immunomodulatory mechanisms. Nevertheless, we could not confirm such an effect modification in our study. However, we cannot exclude that this lack of effect modification was due to limited power in the stratified analyses.

Although animal data favour a causal role (8, 17), it is still possible that the association between adipokine levels and sensitization is because of chance or bias. In addition, as our study is only cross-sectional, we can only speculate about causality. However, if such a causal role is assumed, the observed associations and lack of interaction may reflect primarily acute conditions posed by the subjects' present obesity. This acute effect seems to be

independent of immunological memory acquired in early childhood. If these conclusions are valid, our findings would further emphasize the public health impact of the rising prevalence of obesity in young adults. Moreover, it would indicate interventional potential with regard to allergy prevention.

In conclusion, our data demonstrated that – taking obesity into account – increased blood levels of leptin were associated with allergic sensitization. Early childhood farm contact, resulting in a lower overall level of allergic sensitization, did not modify this association in a statistically significant way. These findings suggest that adipokines are involved in the pathways linking obesity and allergic sensitization. Whether this involvement acts independently of childhood farm contact has to be shown in larger studies.

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Acknowledgments

The authors are grateful to Dr. Georg Praml, Bernhard Schwertner, Martina Dutschke, Julia Post, Dr. Ursula Auge, Alexandra König, and Susanne Schelinski for the field work. Special thanks to Vera Ehrenstein and Dr. Rob van Strien for data management and statistical analyses. In addition, we thank Bettina Prüller for her support. We thank the participants for their cooperation.

Financial support

The study has been funded by the Ministry of Social Affairs, Women and Health of Lower Saxony and by the European Union. The authors disclose any financial relationship with a biotechnology and pharmaceutical manufacturer that has an interest in the subject matter or materials discussed in the submitted manuscript.

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