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Role of inflammation markers in the prediction of weight gain and development of obesity in adults – a prospective study

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Abstract

BACKGROUND AND AIMS. There is a growing body of literature confirming the association between inflammation and obesity. Recent research suggests that inflammation may play a role in weight gain. The aim of the study was to analyse whether serum inflammatory markers predict weight gain or development of obesity in a prospective study design.

METHODS AND RESULTS. The baseline study (DILGOM 2007) consists of a population-based sample of 5024 Finnish men and women aged 25-75 years, of whom 3735 participated in the follow-up study in 2014. Baseline data collection included a questionnaire on health behaviour, physical examinations and blood samples including serum high-sensitivity C-Reactive Protein (hs-CRP), Interleukin-1 receptor antagonist (IL-1Ra), Interleukin-6 (IL-6), Tumor Necrosis Factor Alpha (TNF-alpha) and high molecular weight adiponectin (HMW adiponectin). Indicators of obesity were weight, body mass index (BMI), waist circumference and body fat percentage (% body fat). At baseline hs-CRP, IL-1Ra, IL-6, TNF-alpha and HMW adiponectin associated strongly ($p<0.0001$) with obesity indicators. After adjustment for several potential predictors of obesity, hs-CRP and IL-1Ra associated inversely with changes in obesity indicators during the 7-year follow-up. These associations disappeared, however, after further adjustment for baseline BMI. Only HMW adiponectin retained a modest positive association with the change in weight ($p=0.008$), in BMI ($p=0.007$) and in waist circumference ($p=0.002$).

CONCLUSION. These findings suggest that the inflammatory markers, although highly associated with obesity, do not predict weight gain in an adult population. This could translate into inflammation being a result of obesity rather than a contributing factor to it.

Key words:

Inflammation; Obesity; Weight gain; Epidemiology

1. Introduction

Obesity is a major public health challenge in the developed world and increasingly also in the developing world (1,2).

Obesity and being overweight have been associated with low-grade chronic inflammation (3,4,5,6,7,8). Recent research suggest that inflammation may play a role in the process of weight gain in children (9) and in adults (10). It has been a chicken and egg question and no definitive results have been presented as to whether subclinical inflammation actually is a cause or a consequence of obesity. Accordingly, the roles of a variety of inflammation markers as predictors of weight gain are not clear.

Increased levels of markers of inflammation such as high sensitivity C-Reactive Protein (hs-CRP), certain interleukins and Tumor Necrosis Factor Alpha (TNF-alpha) have been linked with metabolic disorders, cardiovascular diseases as well as an increased risk of mortality (11,12,13). Obesity also influences the development of these outcomes. Better understanding of the link between weight gain, obesity and the development of low-grade chronic inflammation could prove useful in addressing these major public health issues.

The aim of the study was to analyse whether subclinical inflammation precedes and predicts obesity. The specific objective was to analyse whether elevated levels of hs-CRP, interleukin-6 (IL-6), interleukin-1 receptor antagonist (IL-1Ra), TNF-alpha and reduced levels of high molecular weight adiponectin (HMW adiponectin) preceded weight gain, increasing body mass index (BMI), increasing waist circumference and increasing body fat percentage.

2. Methods

2.1 Baseline survey and follow-up

The DILGOM (the DIetary, Lifestyle and Genetic determinants of Obesity and Metabolic syndrome) study was conducted as an extension of the National FINRISK 2007 Study in April-May 2007. DILGOM 2007 was the baseline study of a population-based sample of men and women aged 25-75 years living in Finland (n=5024, participation rate 80%). Participants of the baseline study responded to questionnaires, underwent physical examination (including anthropometric measures) by trained nurses and gave blood samples. Detailed study protocols, including the sampling, measurements and blood sample protocols are described in detail elsewhere (14,15). The participant flowchart for the baseline and follow-up study has been published earlier (15) and can be viewed online here: https://media.nature.com/original/nature-assets/ijo/journal/v42/n4/extref/ijo2017278x2.pdf.

Seven years later, all living baseline study participants were invited to participate in the DILGOM follow-up study in 2014 and altogether 3735 participants (response rate 82%) returned the survey questionnaire. A comparison of the characteristics between participants and non-participants has been presented earlier (15).

Participants from the capital metropolitan area and Southwestern Finland attended a health examination (n=1312). During the health examination, trained nurses measured weight, height, waist circumference and body fat percentage. This examination was carried out in the same spring months and following the same standardized protocol as for the baseline examination. Blood samples were drawn from the participants attending the health examination. Participants who were not invited for a health examination (n=2423) reported their current weight, height and waist circumference; the latter was measured by the participants themselves according to detailed written instructions received together with a

measurement tape. The self-reported measurements have been validated against the measurements by trained nurses (16). Body fat percentage (% body fat) was measured with a bioelectrical impedance instrument (TANITA TBF-300MA, Tanita Corporation of America, Inc., Arlington Heights, IL, USA) (17).

Record linkages based on the personal identification code to Finnish National health care registers such as the Hospital Discharge register and the National Causes-of-Death register were used to identify subjects that needed to be excluded from the analysis due to any prevalent or incident disease (detailed under the Design section) relevant to weight change at baseline or during follow-up.

The study plan for the DILGOM baseline examination was approved by the Coordinating Ethical Committee of the Helsinki and Uusimaa Hospital District on 03.04.2007. The decision number is 229/E0/2006. The study plan for the re-examination was approved by the same Ethical Committee on 14.01.2014. The decision number is 332/13/03/00/2013. All participants signed an informed consent.

2.2 Design

We analysed the DILGOM baseline and follow-up data (from the questionnaires, physical examinations and blood samples) to determine whether serum inflammation markers are associated with weight gain and development of obesity in the 7-year-follow-up.

The main outcome measures were changes in weight (in kg), BMI (kg/m^2), waist circumference (cm) and body fat percentage (%-unit) during the 7-year follow-up. The explanatory variables of main interest were inflammatory markers hs-CRP, IL-1Ra, IL-6, and TNF-alpha as well as the anti-inflammatory protein HMW adiponectin.

Participants with established weight-loss causing diseases prevalent at baseline or incident during the 7-year follow-up, such as cancer (excluding ICD10 category C44), hyperthyroidism, HIV and tuberculosis were excluded. Participants who were pregnant either at baseline or at follow-up, were also excluded. In addition, based on visual inspection of the outcome measure distributions, three extreme outliers (one with 40.7kg weight gain, one with 51.6kg weight loss and one with 88.5 cm waist gain during the 7-year follow-up) were excluded. Altogether 366 individuals were excluded from the analyses. In total the study population included 3369 participants.

2.3 Laboratory methods

Concentrations of hs-CRP were measured from frozen serum samples (-70°C) using a latex immunoassay (Sentinel diagnostics, Milan, Italy) on Architect c8000 analyzer (Abbott Laboratories, Abbott Park, IL, USA), interleukin 6 and TNF-alpha concentrations with multiplex sandwich immunoassays (Milliplex High Sensitivity Human Cytokine kit, Millipore, Billerica, MA, USA) and IL-1Ra and high molecular weight adiponectin concentrations using enzyme linked immunosorbent assays (Human IL-1ra/IL-1F3 Quantikine ELISA Kit, R&D Systems, Inc., Minneapolis, MN, USA and Human HMW Adiponectin ELISA kit, Millipore, Billerica, MA, USA) for IL-1Ra and HMW adiponectin, respectively. Hs-CRP measurements of samples drawn at follow-up were conducted using the same method as mentioned above.

2.4 Statistical methods

Means and standard deviations were calculated for normally distributed continuous variables, geometrical means and anti-logs of standard deviations are shown for continuous variables with a skewed distribution and frequencies for categorical variables. Welch Two Sample t-tests were used to compare baseline and follow-up values.

We ran a residual analysis to determine the appropriateness of a linear regression model for hs-CRP, Il1-Ra, IL-6, TNF-alpha and HMW adiponectin. As a result of these analyses, we used log-transformed inflammatory markers in linear regression analysis. To enable comparison between the different inflammation markers, we expressed the associations per one standard deviation (SD) difference in log-transformed concentrations of the inflammation markers.

Generalized linear regression models were applied for analysing cross-sectional and longitudinal associations between the explanatory variables and the obesity indicators. Conventional risk factors for weight change and other relevant baseline characteristics such as age, sex, education, smoking, alcohol consumption, energy intake, physical activity at leisure time and BMI at baseline were adjusted for in multivariable linear regression models. Age, alcohol consumption, energy intake and BMI were used as continuous variables whereas the remaining ones were categorical variables (categories named in table 1). For each analysis, outliers with a difference of more than 3 standard deviations from the mean of the inflammation marker level were excluded from the analysis. The continuous outcome variables used were: change in weight, change in BMI, change in waist circumference and change in % body fat.

We also performed logistic regression for hs-CRP with BMI cut-off points less than 30kg/m^2 and equal to or over 30kg/m^2 as an outcome and for a cut-off point of 10% or more weight gain and less than 10% weight gain during the 7-year follow-up as an outcome. Finally, we performed the linear regression analyses for hs-CRP using only never smokers.

Apart from the baseline characteristics, all results are presented for analyses with women and men combined as there were no differences in the results between the sexes. The analyses were conducted using R 3.4.1 (R Core Team 2017) and RStudio 1.0.153.

3. Results

Baseline and follow-up characteristics are presented for men and women in tables 1 and 2. Average weight change during the 7-year follow-up was 0.70 kg, with a minimum of -31.5 kg and maximum of 31.9 kg. Notably, 10.4% (12.2% in women and 8.3% in men) of the study population gained 10% or more of weight during the 7-year follow-up.

3.1 Cross-sectional analyses

A linear regression analysis, accounting for age and sex, was performed for the inflammation markers with each other, as well as with baseline values of obesity and other related factors (Table 3). Hs-CRP, IL-1Ra, IL-6, TNF-alpha and HMW adiponectin were associated with each obesity measure at baseline ($p<0.0001$). They were generally associated with each other apart from HMW-adiponectin not being associated with IL-6 and TNF-alpha. Hs-CRP, IL-1Ra and IL-6 were also strongly associated with physical activity and level of education.

We tested for any differences in baseline inflammation status between participants who had lost weight and those who had gained weight at follow-up. In linear regression models for these subgroups, after adjusting for age, gender and baseline BMI, we found a modest direct association with the change in weight and IL1-Ra (for both groups) and HMW adiponectin (weight gain group). However, hs-CRP, IL-6 and TNF-alpha were not associated with the change in weight in this subgroup analysis.

3.2 Longitudinal analyses

Hs-CRP and IL-1Ra levels had a modest inverse association with changes in weight, in BMI, in waist circumference and in % body fat (Model 2, $p<0.001$) (Table 4). However, this association disappeared after adjustment for baseline BMI.

In all models, IL-6 and TNF-alpha had largely non-significant inverse associations with each of the outcome variables (change in weight, BMI, waist circumference and % body fat) (Table 4).

HMW adiponectin was associated with a small but statistically significant ($p<0.001$) increase in the changes in weight, BMI and waist circumference in models 1 and 2. These modest changes remained statistically significant after adjustment for baseline BMI (model 3) for changes in weight ($p=0.008$), BMI ($p=0.007$) and waist circumference ($p=0.002$). Although HMW adiponectin had a small association with changes in % body fat in models 1 ($p=0.005$) and 2 ($p=0.014$), the statistical significance did not persist in model 3.

We also ran logistic regression models for hs-CRP with BMI cut-off points at 30kg/m^2 and 10% of weight gain during the 7 years follow-up. However, these analyses produced similar, non-significant results. Furthermore, ex-smokers, current smokers and never smokers were also analysed separately with linear regression models for hs-CRP with no significant difference in results.

In order to establish whether study participants whose weight increased also experienced an increase in their inflammation status, we conducted a subgroup analysis (n=1158) for those that showed increased and those that showed decreased levels of hs-CRP at follow-up. In linear regression models, after adjusting for age, gender and baseline BMI, we found no association with the change in inflammation status, represented by change in hs-CRP, and the change in weight at follow-up.

4. Discussion

This is the first prospective cohort study among adults examining the effects of a versatile panel of inflammation markers on multiple indicators of obesity development, controlling for established confounding factors. Contrary to our expectations, it was lower levels of hs-CRP and IL-1Ra and higher levels of HMW adiponectin that seemed to predict gains in obesity indicators. And after adjusting the multivariate models for baseline BMI, we did not see any significant associations with our outcomes i.e. changes in obesity indicators.

Results from the ARIC study in 2003 suggested that a mild chronic systemic inflammatory state predicted weight gain in people who quit smoking (18). Similarly, significant associations of higher levels of fibrinogen and CRP with large annual weight gain have been shown in new smoking quitters (10). Our results looking at hs-CRP in different smoker categories did not support these findings.

Our findings are of interest because a clear relationship exists between obesity, insulin resistance and type II diabetes. There is also a current understanding that inflammation leads to impaired insulin action, and inflammation has been shown to predict both insulin resistance and type II diabetes (19,20). Although inflammation plays a central role in obesity-induced conditions, its contribution to weight gain or the development of obesity seems to be virtually non-existent.

We are not aware of follow-up studies, which would have carried out repeated measurements of inflammation markers after weight gain. However, studies have examined whether weight loss decreases the levels of inflammation markers. Askarpour and colleagues recently carried out a meta-analysis of studies examining weight reduction and

inflammation after bariatric surgery (21). They showed that, on average, the weight reduction was accompanied by clear decreases in the levels of inflammation markers.

Recent studies exploring the causality between inflammatory markers and obesity indicators seem to be consistent with our findings. A study using a reciprocal Mendelian randomization design to analyze a Danish adult population concluded CRP to be a marker of elevated adiposity rather than a driver of BMI (22). Similarly, recent work using UK biobank data and based on the Mendelian randomization design found chronic inflammation to be a consequence rather than a cause of obesity (23).

4.1 Strengths and limitations

This study has several strengths. A large population-based random sample of 25 to 75-year-old adults, 7-year prospective follow-up, and high participation rate both at baseline and follow-up. Analyses were controlled for traditional risk factors for weight gain, other factors that may affect inflammation marker levels and baseline BMI.

The main limitation was that we could not invite all participants for the physical re-examination. Despite the validation of self-measurements against the nurse measurements at follow-up, there may still be a bias in the reporting of these values. Self-reported smoking status, alcohol use, total energy intake and the level of physical activity may also be biased due to self-reports. Furthermore, the baseline study participants that dropped out from the follow-up, were slightly younger and heavier and had modestly more elevated levels of inflammation markers as compared to the final study population. It is, however, unlikely that the associations between the weight change and inflammation markers would differ substantially between the participants and non-participants of the follow-up study. Finally, although hs-CRP measurements were available from samples of participants who attended

the re-examination at follow-up, we did not have follow-up data on the other inflammation markers.

4.2 Conclusion

Our study suggests that low-grade inflammation, exemplified as increased levels of hs-CRP, IL-1Ra, IL-6, TNF-alpha and decreased levels of HMW adiponectin, does not predict future weight gain or changes in other indicators of obesity. Inflammation seems to be rather a consequence of increase in weight and accumulation of adipose tissue, especially around the waist. Its role amongst many other factors in the complex set of metabolic and cardiovascular disorders, smoking, mental health and other chronic illnesses vis-à-vis obesity remains still somewhat unclear. Further prospective studies using well-defined and professionally measured continuous values as well as solid linkages to health registers will be needed to confirm whether inflammation has any role to play in the development of obesity.

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6. Funding

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7. Competing interests

Prof Salomaa has participated in a conference trip sponsored by Novo Nordisk and received a modest honorarium from the same source for participating in an advisory board meeting. Other authors declare no competing interests.

8. Contributors

KT, VS and PJ designed the study. KT performed the statistical analyses and drafting of manuscripts. SM and KB contributed to the data acquisition. ASH advised in statistical analyses. All co-authors critically revised the manuscript and approved the final version.

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351 **TABLES**

352 **Table 1. Participant characteristics at baseline**

Baseline characteristics	**Women (n= 1 836)**	**Men (n= 1 533)**
Age, years	51.4 ± 13.1	52.5 ± 12.8
Height, cm	162.5 ± 6.2	176.0 ± 6.7
Waist circumference, cm	86.1 ± 13.0	95.8 ± 11.3
Hip circumference, cm	101.3 ± 10.5	100.0 ± 7.2
Smoking, n (%)		
Never smokers	1234 (67.5)	736 (48.2)
Ex-smokers	325 (17.8)	495 (32.5)
Current smokers	270 (14.8)	295 (19.3)
Educational status, n (%)		
Low	577 (31.8)	392 (25.7)
Middle	608 (33.4)	549 (35.9)
High	632 (34.8)	586 (38.4)
Physical activity at leisure time, n (%)		
Low level or no exercise	315 (17.2)	257 (16.8)
Light exercise, at least 4h per week	1025 (56.2)	787 (51.5)
Aerobic exercise, at least 3h per week	474 (26.0)	445 (29.1)
Regular exercise at competitive level	11 (0.6)	39 (2.6)
Total energy intake, MJ/day	9.7 ± 3.2	11.9 ± 4.0
Weekly alcohol intake, g/week	37.2 ± 64	86.9 ± 117
hs-CRP, mg/l	1.17 (1.78)	1.07 (1.51)
IL-1Ra, pg/ml	282 (182)	263 (143)
IL-6, ng/l	2.75 (3.73)	3.08 (4.18)
TNF-alpha, ng/l	5.21 (3.42)	6.03 (3.59)
HMW Adiponectin, ng/ml	4 873 (4548)	2 560 (2630)

353 Results are presented as means (standard deviation) and percentages, except for hs-CRP,

354 Il1Ra, IL-6, TNF-alpha and HMW adiponectin where geometric means (interquartile range

355 IQR) are reported

356

Table 2. Baseline and follow-up values of outcome variables

	Women at baseline (n = 1 836)	Women at follow-up	p value	Men at baseline (n = 1 533)	Men at follow-up	p value
Weight, kg	70.0 ± 13.5	71.0 ± 14.0	0.025	82.9 ± 13.1	83.3 ± 13.4	0.17
Waist circumference, cm	86.1 ± 13.0	88.1 ± 13.2	<0.001	95.8 ± 11.3	98.0 ± 11.0	<0.001
BMI, kg/m^2	26.5 ± 5.2	26.9 ± 5.2	0.035	26.9 ± 3.8	27.1 ± 3.9	0.156
% of body fat	34.2 (10.4)	34.6 (10.5) n = 635	0.213	23.6 (7.9)	23.3 (7.3) n = 478	0.415

Results are presented as means (standard deviation), except for % body fat where geometric mean (interquartile range IQR) is reported; p values represent results of Welch's Two Sample t- tests.

Table 3. Regression analysis (β-coefficients per SD) examining the association of inflammation markers with each other and with baseline values of obesity and related factors, adjusted for age and sex

	hs-CRP	IL-1Ra	IL-6	TNF-α	HMW adiponectin
Height (cm)	-0.008*	-0.008*	-0.005	0.001	0.003
Weight (kg)	0.027**	0.034**	0.008**	0.007**	-0.012**
Waist circumference (cm)	0.035**	0.044**	0.011**	0.009**	-0.017**
Hip circumference (cm)	0.041**	0.051**	0.013**	0.010**	-0.015**
BMI (kg/m^2)	0.090**	0.111**	0.028**	0.022**	-0.040**
% body fat	0.063**	0.077**	0.018**	0.016**	-0.028**
Energy intake (kJ/day)	0.000	0.000	0.000	0.000	0.000
Smoking status	0.073**	0.098**	0.017	0.039	0.004
Educational level	-0.069^	-0.100**	-0.097**	-0.028	-0.011
Physical activity	-0.229**	-0.317**	-0.110**	-0.082*	0.036
Alcohol consumption (g/week)	0.000	0.000	0.000	0.000	0.001**
Systolic blood pressure (mmHg)	0.005**	0.007**	0.003^	0.002	-0.003*
Diastolic blood pressure (mmHg)	0.009**	0.015**	0.003	0.003	-0.007**
Total cholesterol (mmol/l)	0.036	0.007	-0.017	0.002	0.03
HDL (mmol/l)	-0.432**	-0.903**	-0.154^	-0.285**	1.016**
Triglycerides (mmol/l)	0.261**	0.437**	0.057	0.133**	-0.276**
HMW adiponectin (ng/ml)	-0.111**	-0.250**	-0.008	-0.038	-
TNF-α (ng/l)	0.136**	0.172**	0.282**	-	-
IL-6 (ng/l)	0.241**	0.158**	-	-	-
IL-1Ra (pg/ml)	0.451**	-	-	-	-

^<0.01, *<0.001, **<0.0001, n ranges from 3256 to 3348 depending on variable

365 **Table 4. Results (β-coefficients per SD) of linear regression models with outcome variables: change in weight (kg), change in waist**
366 **circumference (cm) and change in % body fat during the 7-year follow-up**

	n	Model 1	p value for model 1	n	Model 2	p value for model 2	n	Model 3	p value for model 3
High-sensitivity C-reactive protein, mg/l									
Change in weight	3 289	-0.3276	<0.001	3 158	-0.334	<0.001	3 157	-0.0167	0.877
Change in BMI	3 289	-0.1195	<0.001	3 158	-0.1236	<0.001	3 157	-0.0091	0.812
Change in waist circumference	3 198	-0.5311	<0.001	3 072	-0.5377	<0.001	3 071	-0.1424	0.274
Change in %body fat	1 095	-0.344	<0.001	1 057	-0.3702	<0.001	1 056	-0.1245	0.264
Interleukin-1Ra, pg/ml									
Change in weight	3 264	-0.3193	<0.001	3 136	-0.3722	<0.001	3 135	-0.0294	0.792
Change in BMI	3 264	-0.1137	<0.001	3 136	-0.1333	<0.001	3 135	-0.0076	0.847
Change in waist circumference	3 174	-0.5934	<0.001	3051	-0.6323	<0.001	3 050	-0.1965	0.149
Change in %body fat	1 092	-0.3501	<0.001	1 055	-0.4005	<0.001	1 054	-0.0619	0.597
Interleukin-6, ng/l									
Change in weight	3 233	-0.1512	0.119	3 103	-0.1166	0.24	3 102	-0.0173	0.862
Change in BMI	3 233	-0.0595	0.147	3 103	-0.0471	0.182	3 102	-0.1105	0.753
Change in waist circumference	3 142	-0.179	0.129	3 017	-0.1316	0.274	3 016	-0.0051	0.966
Change in % body fat	1 081	0.2042	0.048	1 043	0.2152	0.043	1 042	-0.1366	0.192
Tumor necrosis factor alpha, ng/l									
Change in weight	3 274	-0.1939	0.048	3 143	-0.1873	0.061	3 142	-0.1176	0.236
Change in BMI	3 274	-0.0676	0.053	3 143	-0.0655	0.065	3 142	-0.0402	0.255
Change in waist circumference	3 183	-0.1107	0.353	3 057	-0.077	0.525	3 056	0.0063	0.956

Change in % body fat	1 091	-0.0906	0.384	1 053	-0.0888	0.405	1 052	-0.0154	0.883
HMW adiponectin, ng/ml									
Change in weight	3 294	0.5066	<0.001	3 163	0.4565	<0.001	3 162	0.2895	0.008
Change in BMI	3 294	0.1816	<0.001	3 163	0.1652	<0.001	3 162	0.1046	0.007
Change in waist circumference	3 203	0.6726	<0.001	3 077	0.6377	<0.001	3 076	0.4125	0.002
Change in %body fat	1 098	0.3067	0.005	1 060	0.2768	0.014	1 059	0.1401	0.214

Model 1: Adjusted for age and sex; Model 2: Model 1 further adjusted for education status, smoking, weekly alcohol intake, daily total energy intake, leisure time physical activity; Model 3: Model 2 further adjusted for baseline BMI. Inflammation markers were log-transformed for the analysis.