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Circulating adipokines and the protective effects of hyperinsulinemia in inflammatory bowel disease

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Abstract

Objective: Adipokines are fat-derived hormones and cytokines with immune-modulating and metabolic properties. Most of them are associated with insulin resistance. The aim of the present investigation was to evaluate circulating levels of adipokines and glucose homeostasis in patients with inflammatory bowel disease (IBD) and to evaluate possible associations with the course and characteristics of the disease.

Methods: Serum leptin, resistin, visfatin, retinol-binding protein-4, adiponectin, glucose, insulin, and inflammatory parameters were analyzed in 93 patients with inactive IBD (49 with Crohn's disease [CD], 44 with ulcerative colitis [UC]), 35 patients with active IBD (18 with CD, 17 with UC), and 37 age- and body mass index–matched healthy controls. Ninety-two patients were followed for 6 mo.

Results: Leptin was similar in patients with IBD and controls, whereas resistin and visfatin were increased in patients with active disease but not in those in remission. In active and inactive disease, adiponectin was decreased ($P < 0.001$) and retinol-binding protein-4 was increased ($P < 0.001$) compared with controls. About 60% of patients with IBD showed increased levels of insulin, whereas serum glucose remained normal, resulting in increased homeostasis model assessment values in most patients. Hyperinsulinemia was associated with the decrease in adiponectin ($r = -0.572$, $P < 0.001$) and proved to be an independent protective factor for 6-mo maintenance of remission ($P = 0.016$).

Conclusion: IBD led to largely similar alterations in circulating adipokines and hyperinsulinemia in patients with CD and those with UC. The unexpected protective effect of hyperinsulinemia on relapse rate denotes the role of the metabolic–inflammatory response as a modulator in IBD.

Keywords: Crohn's disease; Ulcerative colitis; Adiponectin; Retinol-binding protein-4; Resistin; Visfatin; Leptin; Pathophysiology; Adaptive mechanisms

Introduction

The advent of adipokines changed the paradigm of mammalian adipose tissue from being a pure reservoir of energy to a highly active endocrine tissue. Adipokines are cytokines and hormones with metabolic and immune-modulating properties secreted by adipocytes and the non-adipocyte fraction of adipose tissue [1–3]. Most adipokines are asso-

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ciated with insulin resistance, for instance adiponectin, leptin, retinol-binding protein-4 (RBP-4), and visfatin [4]. Some have a higher association to proinflammatory activities, foremost resistin [5]. Altered levels of adipokines have been reported in obesity [6], metabolic syndrome [7], type 2 diabetes mellitus [8], human immunodeficiency viral lipodystrophy [9], joint diseases [10], end-stage renal failure [11], and liver disease [12,13].

Inflammatory bowel disease (IBD) is associated with alterations in fat mass and fat distribution. Alterations in fat mass include increases of visceral fat mass [14] and subcutaneous adipose tissue [15]. Changes in fat distribution comprise the fat halo sign [16] and creeping fat or fat wrapping, the latter being lipohypertrophies at the intestinal surface seen in Crohn's disease (CD) only [17,18]. The fat halo sign is caused by intramural lipid infiltrations in the bowel wall and can be depicted on computed tomographic scans in CD and ulcerative colitis (UC). These alterations in fat mass and fat distribution were long considered a negligible bystander, but recently it was suggested that they might cause alterations in the secretion of adipokines and thereby play a pivotal role in the inflammatory process [17]. In addition, they may predispose to insulin resistance through the action of adipokines.

Therefore, the aims of the present investigation were to assess circulating levels of adipokines and glucose homeostasis in patients with inactive and active IBD and to compare results with healthy controls. In addition, possible associations to disease characteristics and rate of relapse were to be identified.

Materials and methods

Subjects

The study protocol was approved by the ethics committee of the Charité–Universitätsmedizin Berlin and all subjects gave their informed consent before the start of the study.

Patients

The study involved 128 patients with IBD (67 with CD, 49 women and 18 men, 34 ± 11 y of age; 61 with UC, 40 women and 21 men, 43 ± 13 y of age) 18 to 70 y of age who were recruited at our department between September 2004 and August 2005. Inactive disease was defined as a Crohn's Disease Activity Index (CDAI) score <150 [19] or a Colitis Activity Index (CAI) score <5 [20] for at least 3 mo, and active disease as a CDAI score ≥150 or a CAI score ≥5. The CDAI and CAI are accepted gold standards for the assessment of disease activity in patients with CD and UC, respectively. Clinical data of the patients are presented in Table 1.

Table 1
Patient characteristics

	Inactive IBD	Active IBD
CD		
Patients (*n*)	49	18
CDAI	52 (31, 109)	224 (187, 284)
Disease duration (mo)	84 (50, 180)	72 (23, 159)
Localization (*n*)*		
Upper GI tract	14	6
Jejunum/ileum	12	3
Terminal ileum	37	13
Colon	37	7
Resections (*n*)†		
Patients w/resections	19	7
Jejunum/ileum	7	0
Ileocecum	13	7
Colon	8	2
UC		
Patients (*n*)	44	17
CAI	3 (2, 5)	8 (7, 10)
Disease duration (mo)	96 (35, 150)	96 (72, 129)
Localization (*n*)		
Rectal	2	1
Left-sided colitis	18	6
Pancolitis	24	10
Absolute resections (*n*)	2	1
CD + UC		
Patients (*n*)	93	35
Current medication (*n*)‡		
None	13	4
5-ASA	60	20
Immunosuppressants§	28	15
Topical steroids	14	9
Steroids systemic	5	15
Smoking status (*n*)		
Non-smoker	40	10
Current smoker	28	15
Former smoker	25	10
Previous prednisolone therapy		
Prednisolone ever (*n*)	68	33
Previous 5-y total dose (g)	2.9 (1.5, 3.9)	5.4 (3.3, 11.4)

5-ASA, 5-aminosalicyclic acid; CAI, Colitis Activity Index; CD, Crohn's disease; CDAI, Crohn's Disease Activity Index; GI, gastrointestinal; IBD, inflammatory bowel disease; UC, ulcerative colitis

* Most patients were affected at more than one site.

† Multiple answers possible.

‡ Medication reflects current medication; some patients received more than one drug.

§ Azathioprine, methotrexate, and infliximab.

The diagnosis and extent of disease were confirmed by standard criteria. Exclusion criteria were severe concomitant diseases, chronically active disease (CDAI score >150 over 6 mo despite steroid therapy of at least the equivalent dose of 10 mg of prednisolone or relapse after discontinuation of drug therapy), pregnancy, ostomy, and celiac disease. Actual medication and previous prednisolone therapy (≤5 y) were recorded in all patients.

Data on the 3- and 6-mo relapse rates were prospectively recorded using questionnaires sent to the patients 6 mo after the initial assessment. The patients were informed of the 6-mo follow-up period at the primary assessment and

trained to evaluate their disease activity according to the Harvey Bradshaw Index [21] in CD and the CAI in UC. Patients were rated as "relapsed within 6 mo" when they reported a relapse in the previous 6 mo or were in the active phase at the time of the mailing. Ninety-two patients (73.4%) returned the questionnaires.

Healthy controls

Thirty-seven healthy controls (31 women and 6 men, 39 ± 10 y of age) matched by age and body mass index (BMI) were recruited from the general population of Berlin. Health was defined as absence of acute or chronic disease, no acute or chronic medication, and all standard routine blood parameters within the normal range.

Methods

Biochemical parameters

A venous blood sample (50 mL) was obtained from patients and controls after an overnight fast. Glucose, C-reactive protein (CRP), α_1-acid glycoprotein (AAG), and blood count were analyzed in the routine laboratory. The remaining blood was centrifuged at 6°C and stored at −80°C within 30 min after withdrawal.

Leptin, adiponectin, resistin, interleukin-6, and insulin were analyzed using commercially available human serum adipokine LINCOplex kits (panels A and B) according to the manufacturer's instructions (Millipore, Amsterdam, Netherlands). These analytes were measured in the multiplex bead assay using a Luminex200 system in 96-well plate format. Briefly, serum samples (1:400 dilution for adiponectin and resistin), controls, and serial dilutions of standard were incubated with antibody-immobilized fluorescent beads over night at 4°C. The next day specific detection antibody was added and washed, and the beads incubated with streptavidin-phycoerythrin to quantify the amount of antigen bound to the beads. All measurements included independent quality controls.

Visfatin and RBP-4 were measured according to the manufacturer's instructions using separate commercially available enzyme-linked immunosorbent assays (Phoenix Pharmaceuticals, Belmont, CA, USA).

Insulin resistance was calculated according to the homeostasis model assessment (HOMA) test with the following formula: insulin (microunits per milliliter) × glucose (milligrams per 100 mL)/405, where 1 pg of insulin equals 1/34.2 μU. HOMA values <1 are considered normal and values >2.5 indicate a high probability of insulin resistance.

The determination of the total fatty acid content in plasma was based on an esterification procedure and a subsequent gas–chromatographic analysis of the fatty acid methyl esters as described by Sattler et al. [22]. Coefficients of variation (within-run) for the different fatty acids were 0.38% to 8.28%.

Nutritional assessment and body composition

Height and weight of the patients and controls were measured on the day of assessment, and their BMI was calculated as weight (in kilograms) divided by square height (in meters).

Body composition was assessed with bioelectrical impedance analysis. Bioelectrical impedance analysis was performed in patients and controls as described elsewhere [23] using a Nutriguard M analyzer (Data Input, Darmstadt, Germany) applying alternating electrical current of 800 μA at 50 kHz to measure resistance, reactance, and phase angle α. Body cell mass measured by bioelectrical impedance analysis was calculated as fat-free mass × 0.29 × ln (α) [24] using the formulae for fat-free mass as total body water/0.732 and total body water = 0.69 × height^2/resistance + 0.8 [25]. Measurements were taken in subjects after a 12-h overnight fast, voiding of urine from the bladder, and lying in a supine position for 15 min.

Statistics

Statistical analysis was carried out using SPSS 13 (SPSS Inc., Chicago, IL, USA).

Data are expressed as median (25th percentile, 75th percentile) or mean ± standard deviation. Multiple comparisons were performed by the Kruskal-Wallis test and Mann-Whitney U or chi-square tests were used to analyze differences between two groups. Correlations were calculated using Spearman's rank-order correlation coefficient for non-parametric data. To identify independent influencing factors of the 6-mo relapse rate, multiple logistic regression analysis was used. In Figures 1 to 4 box plots were used for the visual interpretation of the results. Box–whisker plots display the 25th, 50th, and 75th percentiles in the boxes and the minimum and maximum as whiskers, except for extreme values.

An acceptable level of statistical significance was established a priori at $P < 0.05$.

Results

Body composition, plasma fatty acids, and inflammatory markers

Body mass index, fat mass, and body cell mass were comparable in all groups except for a higher BMI value in patients with inactive UC (Table 2).

Total, saturated, and monounsaturated fatty acids were increased in the majority of IBD groups as compared with controls; however, polyunsaturated fatty acids were elevated only in patients with inactive CD. All inflammatory markers were higher in patients with IBD as compared with controls (Table 2), but in inactive disease values were within normal range in more than 70% of patients. Interestingly, results for patients with CD and those with UC were largely similar.

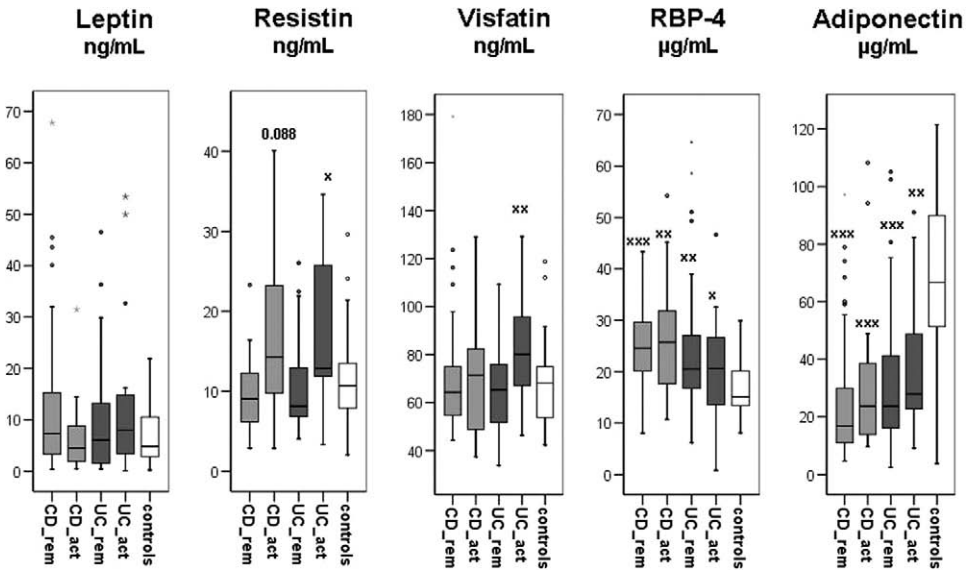

Fig. 1. Circulating levels of adipokines in patients with inflammatory bowel disease and controls ($n = 37$). Leptin was similar in all groups. Resistin and visfatin were increased in active disease only, whereas alterations in RBP-4 and adiponectin were independent of disease activity. We detected no differences between patients with CD and those with UC. $^{x}P < 0.05$, $^{xx}P < 0.01$, $^{xxx}P < 0.001$, patients with inflammatory bowel disease versus controls. CD_act ($n = 18$), active Crohn's disease; CD_rem ($n = 49$), inactive Crohn's disease; RBP-4, retinol-binding protein-4; UC_act ($n = 17$), active ulcerative colitis; UC_rem ($n = 44$), inactive ulcerative colitis.

Adipokines

Circulating levels of leptin were similar in all patient groups as compared with controls (Fig. 1) and fat mass correlated well with leptin in patients with inactive IBD ($r = 0.728$), those with active IBD ($r = 0.755$), and controls ($r = 0.694$) with no difference between CD and UC. Similar values were also seen for leptin expressed per kilogram of fat mass (inactive IBD 0.35 ng/mL [0.18, 0.63]; active IBD 0.33 ng/mL [0.23, 0.57]; control 0.32 ng/mL [0.20, 0.59]). Interestingly, patients with UC who relapsed within 3 mo showed higher leptin values (14.3 ng/mL [5.0, 37.0]) as

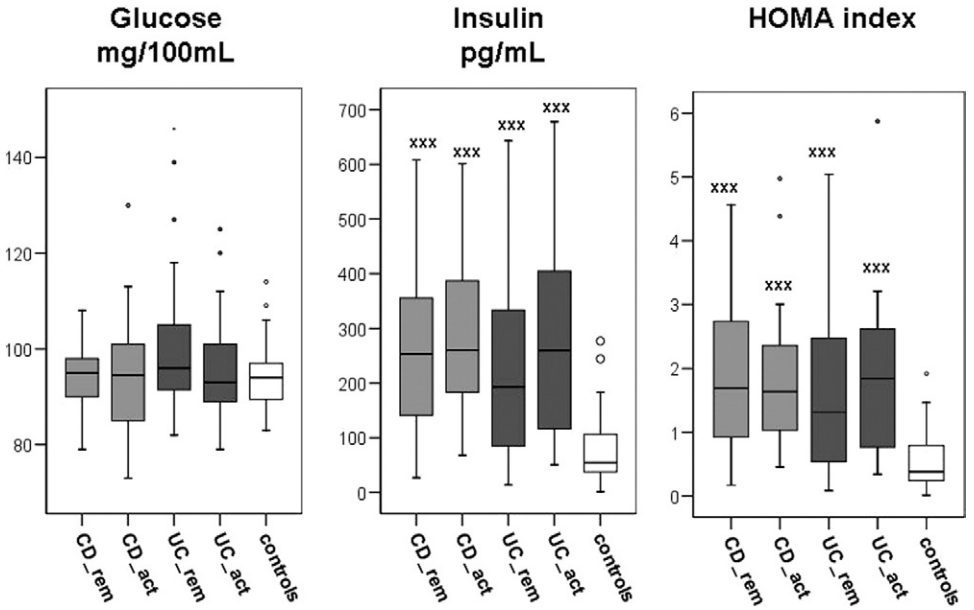

Fig. 2. Glucose, insulin, and insulin resistance in patients with inflammatory bowel disease and controls ($n = 37$). We found normal glucose levels and increased levels of insulin (picograms per milliliter) resulting in increased HOMA values in about 60% of patients. We detected no differences between patients with CD and those with UC. $^{xxx}P < 0.001$, patients with inflammatory bowel disease versus controls. CD_act ($n = 18$), active Crohn's disease; CD_rem ($n = 49$), inactive Crohn's disease; HOMA, homeostasis model assessment; UC_act ($n = 17$), active ulcerative colitis; UC_rem ($n = 44$), inactive ulcerative colitis.

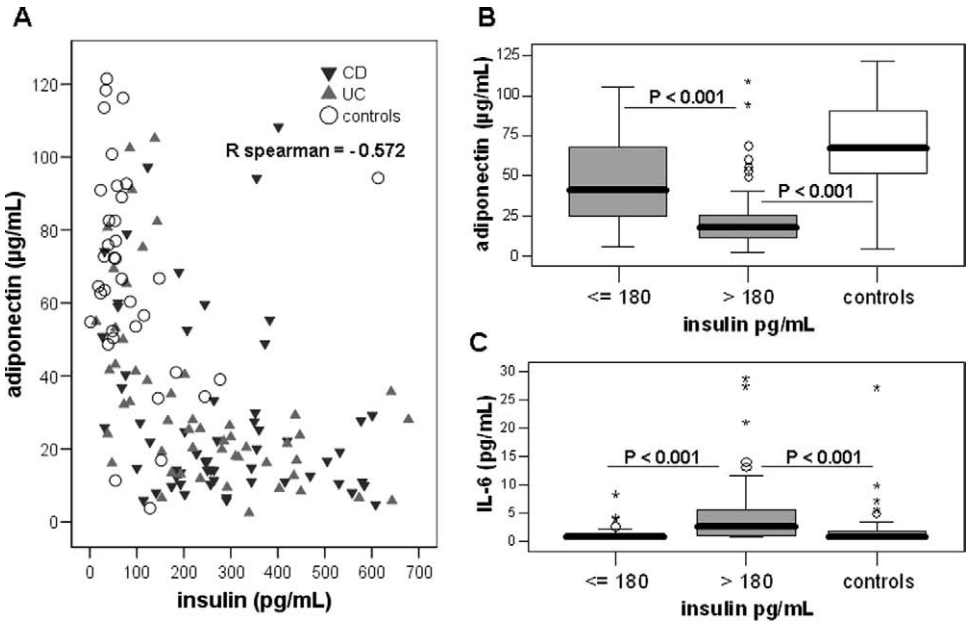

Fig. 3. Associations between hyperinsulinemia and adiponectin and IL-6. (A) Adiponectin was closely associated to insulin but not the remaining adipokines. Hyperinsulinemic patients had lower levels of adiponectin (B) and increased levels of IL-6 (C). Three IL-6 values are not depicted in C (≤180: 49.1 pg/mL, >180: 51 pg/mL, 62 pg/mL). IL-6, interleukin-6.

compared with patients with UC without relapse (6.1 ng/mL [1.4, 9.5], $P = 0.026$). This association was not seen in patients with CD. Neither inflammatory parameters nor actual disease activity correlated with leptin values. Resistin was increased in patients with active disease but not in patients in remission as compared with controls (Fig. 1). Resistin correlated with disease activity scores (CDAI, CAI) and all inflammatory markers except interleukin-6 but not with body fat mass or plasma fatty acids. Visfatin was increased in patients with active UC as compared with controls but unchanged in the remaining patient groups. No associations were seen to inflammatory parameters or body composition. Visfatin was associated with resistin in controls ($r = -0.542$, $P < 0.001$), but not in patients. RBP-4 was increased in all patient groups independent of disease activity (Fig. 1). The increase was associated with an increase in circulating fatty acids ($r = 0.373$, $P < 0.001$), but not with inflammatory parameters or body composition. When patients with active and inactive disease were combined, RBP-4 was significantly higher in those with CD as compared with those with UC (25.0 μg/mL [19.9, 30.4] versus 20.6 μg/mL [15.7, 27.0], $P = 0.033$). Adiponectin was dramatically decreased in all patient groups independent of disease activity (Fig. 1) and correlated with BMI ($r = -0.269$, $P = 0.005$) and RBP-4 ($r = -0.359$, $P < 0.001$) in patients with IBD, but not in controls ($r = 0.079$, $p = 0.646$, and $r = 0.042$, $P = 0.813$, respectively). Adiponectin was associated with body fat mass in female ($r = -0.257$, $P = 0.034$) and male ($r = -0.278$, $P = 0.095$) patients.

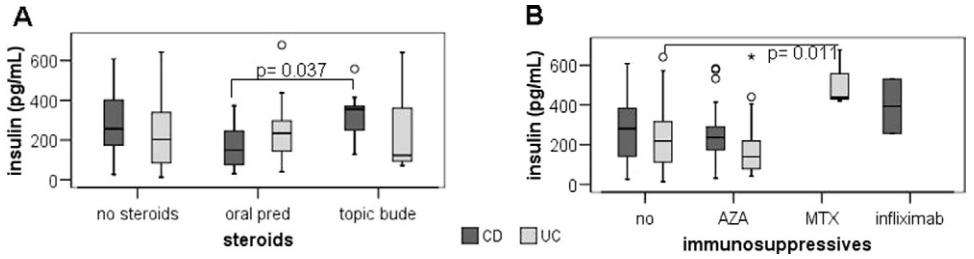

Fig. 4. Insulin and current medication. (A) Insulin levels were similar in all subgroups of patients receiving steroids as compared with patients not receiving steroids. However, insulin was higher in patients with CD on topic budesonide therapy than in patients with CD receiving prednisolone. Because both subgroups were small (prednisolone, $n = 7$, versus budesonide, $n = 11$) these results should be interpreted cautiously. (B) Most patients on immunosuppressive therapy received AZA ($n = 37$). Only three patients with UC and two with CD were on MTX and infliximab therapy. Therefore, the statistically relevant increase of insulin levels in MTX-treated patients needs further evaluation. AZA, azathioprine; CD, Crohn's disease; MTX, methotrexate; pred, prednisolone; topic bude, topical budesonide; UC, ulcerative colitis.

Table 2
Body composition, fatty acids, and inflammatory markers*

	Inactive IBD		Active IBD		Healthy controls (n = 37)
	CD (n = 49)	UC (n = 44)	CD (n = 18)	UC (n = 17)	
Body composition					
Age (y)	36 (27, 45)	42[‖] (30, 56)	32 (26, 43)	42 (33, 52)	39 (30, 46)
Women (%)	74%	64%	72%	71%	84%
BMI (kg/m^2)	22.0 (20.3, 26.1)	24.2[†] (21.0, 27.0)	20.5 (18.8, 24.3)	22.3 (20.2, 24.9)	22.3 (20.7, 24.2)
Fat mass (%)	29.2 (23.1, 34.7)	29.0 (23.8, 35.6)	26.6 (20.4, 32.5)	31.1 (22.4, 36.1)	25.8 (22.2, 29.9)
Body cell mass (%)	35.3 (32.3, 41.0)	35.3 (32.7, 39.2)	36.1 (34.2, 40.1)	35.3 (32.4, 39.8)	37.7 (35.0, 40.4)
Plasma FA (μmol/L)					
Total FA	10354[†] (8902, 11200)	10519[‡] (9403, 11794)	11225[†] (9353, 12857)	10195 (8493, 12279)	9373 (8237, 10570)
Saturated FA	3856[‡] (3311, 4522)	3790[†] (3388, 4289)	4280[‡] (3394, 5308)	3735 (3010, 4554)	2869 (2329, 3370)
Monounsaturated FA	2698[§] (2160, 3290)	2513[§] (2339, 2916)	3050[§] (2417, 3711)	2658[†] (2102, 3154)	2029 (1842, 2433)
Polyunsaturated FA	3593 (3189, 4103)	4127[†‖] (3454, 4558)	3716 (3145, 4106)	3599 (3190, 4646)	3743 (3099, 4135)
Inflammatory parameters					
CRP (<5 mg/L)	1.8[‡] (0.9, 5.0)	2.1[‡] (0.6, 6.2)	12[§] (3.5, 28)	5.0[‡] (0.7, 17.8)	0.8 (0.5, 1.6)
AAG (<1200 mg/L)	820[§] (630, 1000)	725[†] (588, 925)	1210[§] (920, 2040)	1180[§] (885, 1315)	640 (515, 750)
IL-6 (<5 pg/L)	1.32[†] (0.64, 3.68)	1.31 (0.64, 4.24)	4.57[‡] (1.42, 7.23)	1.33[†] (0.91, 3.45)	0.64 (0.64, 2.0)

AAG, α_1-acid glycoprotein; BMI, body mass index; CD, Crohn's disease; CRP, C-reactive protein; FA, fatty acids; IBD, inflammatory bowel disease; IL-6, interleukin-6; UC, ulcerative colitis

* Results are expressed as median (25th percentile, 75th percentile); statistical significance was calculated with the Mann-Whitney-U test.

[†] $P < 0.05$, IBD versus control.

[‡] $P < 0.01$, IBD versus control.

[§] $P < 0.001$, IBD versus control.

[‖] $P < 0.05$, CD versus UC.

Disease duration, localization, type of disease, and actual medication or previous use and dose of prednisolone exerted no influences on adipokines except for higher levels of RBP-4 in patients with CD and intestinal resections (n = 26) as compared with patients with CD without resections (n = 41, 27.0 μg/mL [20.8, 34.4] versus 23.4 μg/mL [18.4, 26.5], P = 0.039).

Insulin, glucose, and insulin resistance (HOMA values)

Irrespective of CD or UC, patients with IBD showed increased levels of insulin (picograms per milliliter), whereas serum glucose (milligrams per deciliter) remained normal, resulting in increased HOMA values in about 60% of patients (Fig. 2). Insulin values were strongly associated with HOMA values (r = 0.987, $P < 0.001$), confirming that hyperinsulinemia alone and without contribution from glucose was responsible for the increases in the HOMA index. Insulin correlated positively with body fat mass (r = 0.367, $P < 0.001$) and plasma fatty acids (r = 0.251, P = 0.008) in patients with IBD, but not in controls. Among the adipokines, only adiponectin correlated with insulin (r = −0.572, $P < 0.001$; Fig. 3A) in patients with IBD (r = −0.449, $P < 0.001$) and controls (r = −0.349, $P < 0.037$). Adiponectin was normal in patients with insulin levels ≤180 pg/mL (Fig. 3B) and significantly decreased in patients with insulin levels >180 pg/mL. Hyperinsulinemia was further associated with increased levels of interleukin-6 (Fig. 3C).

Duration, severity, and type of disease, localization, or cumulative previous 5-y prednisolone use were not associated with insulin levels or HOMA. Regarding current medication, we found higher insulin levels in patients with CD receiving topical budesonide and in patients with UC on methotrexate treatment. However, these results must be interpreted very cautiously because groups were small in these specific subgroups (Fig. 4).

Univariate analyses of potential risk factors for disease relapse revealed a significant association with insulin (P = 0.006) and HOMA (P = 0.017) values, but no correlation to adipokines or inflammatory parameters. Hyperinsulinemic patients relapsed less often than normoinsulinemic patients with IBD (Fig. 5). As known from previous studies, smoking exerted significant deleterious effects on the rate of relapse in patients with CD (P = 0.026) but not in those with UC.

Multivariate analysis confirmed hyperinsulinemia as an independent factor for relapse protection (Table 3). Subgroup analysis resulted in a significant protective effect in CD and a less pronounced effect in UC (Table 3). The less pronounced effect in patients with UC was due to the attenuation of the positive effect in active UC (Fig. 5).

We also performed the multivariate analysis for HOMA index and could confirm the disease protective effect (P = 0.016). However, the effect was more pronounced in patients with HOMA values between 1 and 2.5 (P = 0.005, odds ratio 0.169, 95% confidence interval 0.049–0.588) than in patients with higher HOMA values (P = 0.083, odds ratio 0.330, 95% confidence interval 0.094–1.156).

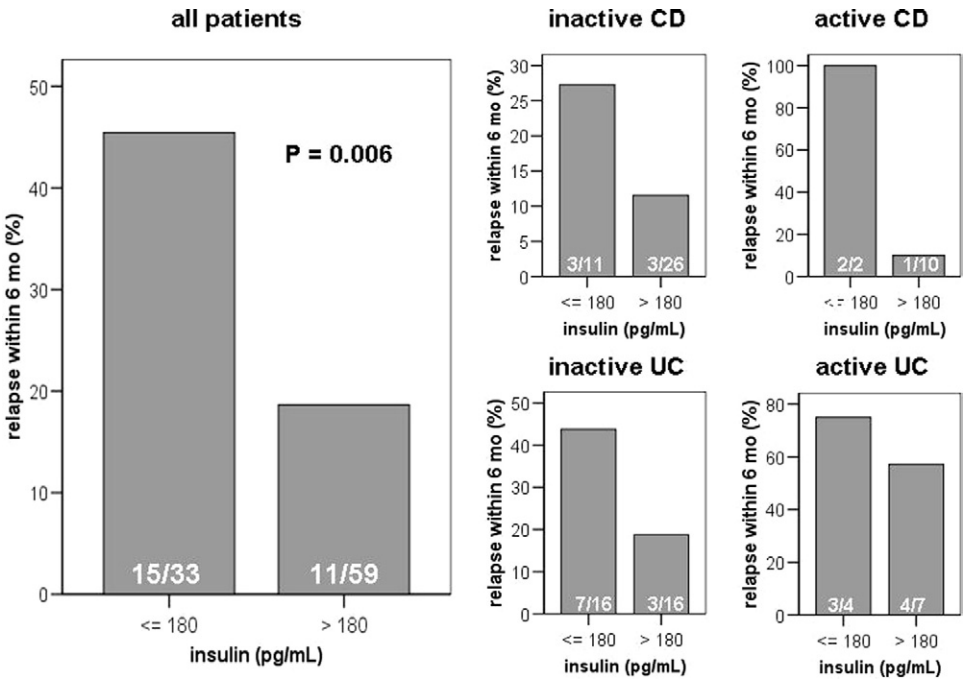

Fig. 5. Hyperinsulinemia and relapse. Patients with increased insulin values relapsed less often than patients with inflammatory bowel disease and normal insulin values. This was true for all subgroups, but reached statistical significance only in the combined group of all patients. The positive effect was less pronounced in active UC. CD, Crohn's disease; UC, ulcerative colitis.

Discussion

In the present study we investigated five circulating adipokines (leptin, resistin, visfatin, RBP-4, and adiponectin) and glucose homeostasis in patients with inactive and active IBD. We observed alterations in serum adipokine levels and increased levels of insulin that were largely similar in patients with CD and those with UC. However, the most striking result was the association between dramatically decreased adiponectin levels and hyperinsulinemia. Interestingly, hyperinsulinemia seemed to have a relapse protective effect.

Only a few previous studies have investigated adipokines in IBD [26–30] and none of the studies included insulin (resistance) or effects on the course of disease as a possible implication of altered adipokines. Previous studies have shown increased expression of leptin in the colon [26] and in creeping fat [29], increased secretion of adiponectin [29], and increased adiponectin mRNA levels in creeping fat [30], whereas expression levels of resistin and adiponectin receptors were normal [29]. Two studies investigated circulating levels of adipokines. These reported normal [28] or decreased [27] levels of leptin and increased levels of adiponectin and resistin [27]. To our knowledge, visfatin and RBP-4 have never been evaluated previously in patients with IBD.

Leptin was normal in our patients, but subgroup analysis revealed increased values in patients with UC who developed an acute flare within 3 mo after the assessment. This might be taken in context to previous findings, where leptin expression in colonic epithelial cells in IBD induced further epithelial wall damage and neutrophil infiltration [26]. Resistin in our study was elevated in active disease but not in patients with disease remission. It was associated with inflammatory parameters and disease activity. Resistin is known to act in a proinflammatory manner through activation of nuclear factor-κB inflammatory pathways [3]. Similarly, visfatin was shown to activate human leukocytes and to induce cytokine production [31]. RBP-4 was increased in all patients groups independent of disease activity. RBP-4 shows the highest correlation to visceral fat mass among all adipokines [32] and is normally closely linked to insulin resistance in humans [33]. In our patients, RBP-4 values were higher in those with CD than with UC, which is in

Table 3
Regression analysis for hyperinsulinemia as an independent protective factor for 6-mo relapse rate

Insulin ≤180 versus >180 pg/L	Univariate	Multivariate*	Odds ratio	95% Confidence interval
	P	*P**		
All (n = 92)	0.006	0.009	0.120	0.025–0.583
CD (n = 49)	0.042	0.027	0.108	0.015–0.778
UC (n = 43)	0.279	0.075	0.232	0.047–1.157

CD, Crohn's disease; UC, ulcerative colitis

* Multivariate analysis after adjustment for age, sex, body mass index, smoking, percentage of body fat, adiponectin, leptin, resistin, interleukin-6, and serum fatty acids.

accordance with the observation of increased mesenteric fat accumulation in CD only [17,18]. However, its expected association with HOMA values was not evident. In contrast to previous studies [27], adiponectin was dramatically decreased in our patients independent of disease activity. Adiponectin is expressed mainly in the subcutaneous adipose tissue and is inversely associated with subcutaneous but not with visceral fat mass. The literature also reports that adiponectin is inversely associated with insulin resistance [34,35], which was true for HOMA values in our patients.

More than 60% of our patients with IBD had increased insulin values, resulting in increased HOMA values, whereas fasting glucose was normal. Glucose metabolism is not well investigated in patients with IBD, presumably because oral glucose tolerance tests and euglycemic insulin clamps have resulted in normal basal or stimulated glucose levels in all previous studies [36–40]. Interestingly, one of these studies [36] also reported increased basal and stimulated insulin levels in patients with CD. In this study, Bregenzer et al. [36] concluded that hyperinsulinemia is due to an upregulation of the enteropancreatic axis, resulting in increased β-cell activity.

In our patients with IBD, hyperinsulinemia proved to be an independent protective factor for the maintenance of remission. Consequentially, increased HOMA values resulted in being protective for the course of disease. HOMA values, however, should be interpreted only cautiously as a marker of insulin resistance in our patients, because increased HOMA values were due to hyperinsulinemia without contribution from glucose. This is in accordance with the results of Bregenzer et al. [36] who also reported hyperinsulinemia with normal fasting and oral glucose tolerance tested glucose levels in their patients with CD. We agree with them on the condition that HOMA should not be interpreted as an indicator of a prediabetic state. Interestingly, and in accordance with our results, Bregenzer et al. [36] casually mentioned that "patients with active disease showed significantly ($P = 0.01$) lower values for HOMA (*sic*, insulin) than patients with inactive disease." This could be another hint that normoinsulinemic patients do indeed relapse more often.

In patients with IBD, the association of insulin secretion with rate of relapse has, to our knowledge, never been investigated before. There are some questions left that we could not answer with our investigation but that might be of interest for future research:

First, is hyperinsulinemia due to medical therapy or is it simply a regulative step of the organism allowing high insulin levels without causing hypoglycemia? Insulin is known to exert protein-anabolic actions [41] and anti-inflammatory effects [42]. Both effects could be considered beneficial in chronic inflammatory diseases such as IBD.

Second, are the alterations in fat mass and fat distribution seen in IBD an innocent bystander of the disease, or are these changes deleterious? Or are they even disease protective as previously suggested [3], which would be in accordance with our results? When is the onset of changes in fat mass and fat distribution in patients with IBD? There are hints that the changes appear very early in the course of IBD, and it might well be that they preset the actual outbreak of disease.

Third, are the changes in adiponectin the cause or effect of increased insulin secretion?

From the evolutionary standpoint, the functional units that control key metabolic and immune functions (immune and blood cells, liver, adipose tissue) have evolved from common ancestral structures, as Hotamisligil [6] pointed out recently. For instance, in ancient insects, the adipose tissue, liver, and hematopoietic system were organized in one functional unit. This developmental heritage may underlie the highly overlapping biological repertoire of these organs. Therefore, it is possible to imagine a situation in which common or overlapping pathways regulate metabolic and immune functions through common key regulatory molecules and signaling systems. A closely linked configuration and coordinated regulation of metabolic and immune responses is likely to be advantageous in certain conditions. During the metabolic–inflammatory response of the organism to injury, the adipokine system provides neuroendocrine signals from body fat for modulation of metabolic and immunological activities to save the physiologic and anatomic integrity of the individual [13], which might cause seemingly paradoxical results in chronic inflammatory conditions.

The present study is limited by the non-compliance of 26.6% of patients in returning the follow-up sheets. Furthermore, among our patients with inactive IBD estimated by the CDAI or CAI, a proportion of patients displayed moderately elevated CRP or AAG levels. However, these markers did not correlate with measurements of insulin, adiponectin, leptin, visfatin, or RBP-4 in our patients. Only resistin was associated with CRP ($r = 0.408$) and AAG ($r = 0.585$). Elevation of CRP and AAG in clinically quiescent IBD is a well-known but unsolved phenomenon even found in patients with endoscopic remission [43]. Thus we cannot exclude that these patients have more active disease than displayed by the CDAI or CAI, but in our study it did not affect the results. We decided a priori to exclude patients with chronically active disease on prednisolone treatment because the literature indicated that chronic exposure to oral prednisolone would increase body fat mass [44,45]. Increased body fat mass could cause alterations in the secretion of adipokines and would thus be a confounding variable. Nevertheless, it would be interesting to perform a future study selectively on that group of patients with chronically active IBD.

Adipokine and insulin concentrations were not affected by disease localization. This is in agreement with the study by Karmiris et al. [27]. However, the number of patients in our study with limited disease, e.g., proctitis, was small. These observations should be thus interpreted carefully,

because we cannot exclude that adipokines and insulin behave differently in limited disease.

Conclusion

Inflammatory bowel disease was associated with alterations in circulating adipokines and insulin, which were largely similar in CD and UC, despite the differences in immunopathogenesis known for these disease entities. In addition, we found evidence that the neurohumoral pattern, especially insulin secretion, affected the degree of inflammatory activity or remission in IBD. This interface between inflammatory and metabolic mediators supports the close link between inflammation and metabolic pathways as recently highlighted by Hotamisligil [6]. A better understanding of the metabolic and inflammatory responses may lead to innovative new therapy strategies in IBD and probably in various other diseases. Further studies are required to confirm our results and to elucidate the underlying mechanisms.

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