gastroenterology insights

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Increased Serum Apelin Levels in Patients with Inflammatory Bowel Disease

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Abstract: Apelin has been implicated in the pathogenesis of several chronic inflammatory diseases through mechanisms related to endothelial cells dysfunction. There is evidence of increased apelin levels in mesenteric adipose tissue and colonic epithelium in patients with inflammatory bowel disease (IBD), but their significance remains unclear. We aimed to measure serum apelin (SA) levels in patients with IBD and to evaluate an association with disease characteristics. SA levels of 104 IBD patients and age and sex matched healthy controls (HCs) in a 1:1 ratio were compared. SA-13 levels were measured using an enzyme-linked immunosorbent assay (ELISA). Mean SA levels were increased in IBD patients compared to HCs (1996.29 ± 1592.96 pg/mL vs. 1552.99 ± 809.64 pg/mL, $p = 0.012$). Both patients without and with cardiovascular disease (CVD) had increased SA levels (2076.44 ± 1714.74 pg/mL vs. 1525.75 ± 818.74 pg/mL, $p = 0.011$ and 1743.01 ± 1116.26 pg/mL vs. 1283.92 ± 726.85 pg/mL, $p = 0.035$, respectively). An inverse association between mean SA levels and a history of musculoskeletal extraintestinal manifestations in the subgroup of IBD patients without CVD was found ($p = 0.043$). The present study—the first to evaluate SA levels in patients with IBD—showed that IBD patients have higher levels of SA compared to HCs. The potential role of SA in IBD merits further investigation in larger studies.

Keywords: apelin; adipokines; inflammatory bowel disease

Citation: Mantaka, A.; Kalyvianaki, K.; Kastritsi, O.; Kampa, M.; Koutroubakis, I.E. Increased Serum Apelin Levels in Patients with Inflammatory Bowel Disease. *Gastroenterol. Insights* **2024**, *15*, 255–265. https://doi.org/10.3390/gastroent15020019

Academic Editor: Toshimi Chiba

Received: 27 January 2024
Revised: 16 March 2024
Accepted: 30 March 2024
Published: 8 April 2024

1. Introduction

Adipokines participate in various biological pathways, including energy metabolism, inflammation, cardiovascular function, and cancer [1,2]. Apelin was identified in 1998 as an adipokine secreted from visceral fat. It is widely expressed in the human organism, playing a key role in the regulation of endothelial cell (EC) function, promoting EC proliferation, migration, and angiogenesis [3–5]. A growing body of research has been focused on the association between apelin and its receptor (APJ) system with EC dysfunction-related chronic inflammatory diseases such as atherosclerosis, obesity, and rheumatoid arthritis, as well as with the regulation of tumor growth and metastasis in several kind of cancers [6–8].

Recently, lower plasma apelin levels have been observed among patients with cardiovascular diseases (CVDs), such as acute or chronic coronary artery disease, hypertension, cardiac failure, and early rheumatoid arthritis, compared to matched healthy controls [9–12]. On the other hand, increased plasma apelin levels were found in obese patients, type 2 diabetes, asthma, and several types of obesity and non-obesity related cancer [13–17]. The mechanisms behind these different associations are not fully understood.

Only a few studies have investigated the role of apelin in inflammatory bowel disease (IBD). Mesenteric adipose tissue (MAT) that migrates around the intestine, known as creeping fat, has been suggested as a key modulating factor in promoting colon injury [18].

The increased expression of proinflammatory cytokines in creeping fat, including peroxisome proliferator-activated receptor (PPAR)-γ and TNF-α, is associated with transmural inflammation, fibrosis, and strictures in Crohn's Disease (CD) [19,20]. Increased expression of apelin in the MAT and colonic epithelium of IBD patients has been reported [21]. The administration of apelin-13 in models of experimental colitis led to decreased disease activity and enhanced intestinal epithelium repair mechanisms [21–23].

Taking into consideration that data on the role of serum apelin (SA) in IBD are lacking, we aimed to measure SA levels in patients with ulcerative colitis (UC) and CD and to evaluate an association with disease characteristics.

2. Materials and Methods

2.1. Study Population

Consecutive patients with a diagnosis of IBD who are followed at the IBD outpatient clinic of the University Hospital of Heraklion, a referral center for IBD in South Greece, were included. Serum samples were collected between October 2019 and August 2021 at patients' regular visits. Inclusion criteria for the study were as follows: (a) established IBD diagnosis since the last 6 months, (b) no history of past or present malignancy, chronic liver or kidney disease, or pregnancy, (c) written informed consent for study participation and blood sampling. After the declaration of the COVID-19 pandemic on 11 March 2020, all patients were tested for SARS-CoV-2 at enrollment. Patients or controls testing positive for or having a history of a recent infection with SARS-CoV-2 in the last 3 months were excluded.

All IBD patients were interviewed during the study period and completed questionnaires to ascertain (a) demographics and lifestyle factors including age, sex, body mass index (BMI), and smoking status (ever or never a smoker); (b) history of established CVD (any lifetime history of myocardial infarction, angina, asymptomatic chronic coronary syndrome, ischemic stroke, hemorrhagic stroke, transient ischemic attack, peripheral artery disease, or heart failure of non-ischemic etiology); or (c) any other classical CVD risk factor including diagnosis of hypertension or taking antihypertensive medications [24], diabetes mellitus [25], dyslipidemia, or taking lipid lowering medications [26].

IBD phenotype, age at IBD diagnosis, disease duration, Montreal classification, IBD treatment including biologics, steroid use at least once for flare, immunomodulators including azathioprine or methotrexate, history of IBD related surgery, history of musculoskeletal extraintestinal manifestations (peripheral arthritis, sacroiliitis, ankylosing spondylitis) and other concurrent autoimmune diseases including systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), psoriasis, primary sclerosing cholangitis, and pyoderma gangrenosum, etc. were recorded. Clinical activity was assessed the day of the interview using the Harvey Bradshaw Index (HBI) for CD patients with an HBI $\leq$ 4 representing clinical remission, and the Simple Colitis Clinical Activity Index (SCCAI) for UC, with SCCAI $\leq$ 2 defined as an absence of clinical disease activity [27,28]. Recent endoscopy records, if available for $\pm$3 months from the date that the serum sample was obtained for apelin measurement, were included in the analysis. Moderate-to-severe endoscopic activity was considered as a Mayo score II and III for UC and the presence of ulcers in one or more parts of colon or terminal ileum for CD. CRP levels at $\pm$7 days of serum apelin sampling were also recorded.

A control group of healthy non-IBD blood donors was included. Controls were $\pm$5 years from the IBD patients and were sex matched to them. Controls selection was based on the criteria of the Greek National Center for Blood Donation. Moreover, the cases with a diagnosis of any autoimmune disease or having risk factors for CVD including hypertension, diabetes mellitus, dyslipidemia, obesity (BMI > 30), and chronic renal failure were excluded. The same gastroenterologist performed interviews and serum sampling on the control group at their visit. Informed consent was obtained from all healthy controls.

2.2. Measurement of Serum Apelin Levels

Quantitative detection of apelin (apelin-13) in serum was performed using a Human APLN (Apelin) ELISA Kit (Wuhan Fine Biological Technology Co., Ltd., C6-323 Biolake, No. 666 Gaoxin AVE. Eastlake High-tech Development District, Wuhan, Hubei, China), following the manufacturer's instructions. More specifically, human serum samples were collected from whole blood, diluted 1:2 with the kit's sample/standard dilution buffer and were placed on the APLN pre-coated plate alongside the standard solutions and control (zero) samples, in duplicate. Biotin-labeled antibody (1:100) was added to each sample for 45 min, at 37 °C. The plate was then washed, followed by HRP-Streptavidin conjugate (1:100) incubation for 30 min at 37 °C. After multiple washes, equilibrated TMB substrate was added to each well and the plate was incubated at 37 °C in the dark for approximately 15 min. Stop solution was then added to the samples, in the same order in which TMB substrate was previously added, and the absorbance (O.D.) was measured at 450 nm using a microplate reader (Multiskan FC, Thermo Scientific). Results were calculated using the OriginLab software, Origin 8.5.1, by generating the standard curve and determining the concentration (pg/mL) of each serum sample multiplied by the dilution factor. Serum apelin-13 levels were measured using ELISA (Wuhan Fine Biological Technology Co., Ltd.).

The protocol of the study was approved by the institutional review board of the University Hospital of Heraklion. This study was supported by a grant from the Hellenic Society of Gastroenterology (protocol number 7597/10-11-2017).

2.3. Statistical Analysis

Comparisons were made 1:1 among all IBD patients and controls. IBD patients with a concurrent diagnosis of CVD were compared 1:1 to ±5 years age- and sex-matched IBD patients without CVD and 1:2 to ±5 years age- and sex-matched controls. IBD patients without a CVD diagnosis were compared 1:1 to their ±5 years age- and sex-matched controls.

Continuous and categorical variables were compared using Student's T-test and Fisher's exact probability test or the $\chi 2$ test, respectively. A p-value of <0.05 was considered statistically significant. Multivariate analysis of covariance (MANCOVA) was used to determine whether there are any significant differences between mean apelin levels and categorical and non-categorical variables related to IBD characteristics and any CVD cofounding factors. Correlations between variables were tested with Spearman's Rho correlation test for non-normally distributed variables. Statistical analyses were performed, using the SPSS software package (version 24, SPSS Inc., Chicago, IL, USA).

3. Results

Overall, SA levels were measured in 104 IBD patients, 56.7% with CD, with a mean disease duration of 15.31 ± 10.08 years and with 61.5% on biologic treatment. The demographics, disease characteristics, endoscopic activity, IBD treatment, and comorbidities of the patients are presented in Table 1.

Table 1. Demographics and disease characteristics of 104 IBD patients included in the study.

	IBD Patients N = 104
Mean age at diagnosis (±SD)	42.19 ± 14.25
Mean age at study entry (±SD)	56.48 ± 11.24
Duration of IBD (years)	15.31 ± 10.08
Female Gender, N (%)	30 (28.8%)
Mean BMI (kg/m^2)	27.82 ± 4.99
Obesity (BMI > 30)	31 (29.8%)
Ever smokers/never smokers	77 (74%)/27 (26%)
UC/CD, N (%)	45 (43.3%)/59 (56.7%)
CD location, N (%) (N = 59) L1/L2/L3	26 (44.1%)/7 (11.8%)/26 (44.1%)
CD behavior, N (%) (N = 59) B1/B2, B3	35 (59.3%)/24 (40.7%)
Perianal CD, N (%) (N = 59)	13 (22%)
E1, E2/E3 (**UC**), **N (%)** (N = 45)	21 (13.6%)/24 (55.9%)
IBD related surgery, N (%)	19 (18.3%)
Biologics, N (%)	64 (61.5%)
Steroids, N (%)	86 (82.7%)
Immunomodulators, N (%)	72 (69.2%)
Extraintestinal (musculoskeletal), N (%)	37 (35.6%)
CVD history, N (%)	25 (24%)
Hypertension, N (%)	41 (39.4%)
Diabetes Mellitus, N (%)	11 (10.6%)
Statins. N (%)	30 (28.8%)
Moderate-severe endoscopic activity, N = 98 (%)	36 (36.7%)
Elevated CRP	34 (32.7%)

IBD, Inflammatory bowel disease; SD, standard deviation; BMI, body mass index; UC, ulcerative colitis; CD, Crohn's Disease; CVD, cardiovascular disease; CRP, C-reactive protein.

3.1. SA in IBD Patients

Mean SA levels were significantly higher in IBD patients compared to matched healthy controls (1996.29 ± 1592.96 pg/mL vs. 1552.99 ± 809.64 pg/mL, $p = 0.012$). Higher mean SA levels were also found in male IBD patients than in the matched male controls (2015.25 ± 1571.94 pg/mL vs. 1560.61 ± 775.25 pg/mL, $p = 0.027$) (Table 2). No difference in mean SA levels between UC and CD patients was found (p = NS). A subgroup analysis by disease characteristics, IBD clinical activity or endoscopic activity scores, IBD treatments, or comorbidities showed no differences, as seen in Table 2.

Multivariate analysis did not find any statistically significant association between SA levels and disease characteristics such as IBD type ($F = (3, 88) = 0.316$, $p = 0.576$), disease duration ($F = (1, 88) = 0.639$, $p = 0.426$), UC extent ($F = (3, 88) = 1.722$, $p = 0.168$), CD location (F = (2, 88) = 0957, $p = 0.388$), CD behavior ($F = (2, 88) = 0.633$, $p = 0.533$) or perianal disease ($F = (1, 88) = 0.002$, $p = 0.963$), use of biologics ($F = (1, 88) = 0.244$, $p = 0.623$), use of immunomodulators ($F = (1, 88) = 2.482$, $p = 0.119$), history of IBD-related surgery ($F = (1, 88) = 0.006$, $p = 0.94$), and musculoskeletal EIMs ($F = (1, 88) = 0.016$, $p = 0.9$). On the other hand, smoking history was found to be the only factor independently associated with SA levels among IBD patients ($F = (69, 125) = 1.890$, $p < 0.001$), with a moderate effect size (partial eta squared 0.511).

A weak negative correlation between age at study entry and SA levels in the all-IBD patients' cohort was observed ($r = -0.214$, $p = 0.002$).

Table 2. Univariate analysis for mean apelin levels among different study groups, different IBD treatments, and CVD risk factors among all IBD patients and their matched controls.

	Mean Apelin (±SD) (pg/mL)	*p* Value
IBD patients/controls	1996.29 ± 1592.96/1552.99 ± 809.64	**0.012**
UC/CD	2060.58 ± 1564.07/1947.26 ± 1626.28	0.721
Female (all)/male (all)	1741.86 ± 1347.35/1787.93 ± 1256.01	0.815
IBD female/control female	1949.54 ± 1670.12/1534.19 ± 902.68	0.236
IBD female/IBD male	1949.54 ± 1670.12/2015.25 ± 1571.94	0.850
IBD male/control male	2015.25 ± 1571.94/1560.61 ± 775.25	**0.027**
UC extent (E1 + E2)/(E3)	1694.87 ± 1102.82/2373.19 ± 2653.62	0.144
CD Montreal L1/CD Montreal L2, L3	1944.29 ± 1270.13/1949.60 ± 1879.71	0.990
CD Montreal B1/CD Montreal B2 + B3	1868.90 ± 1177.80/2061.53 ± 2143.78	0.659
CD perianal/CD no perianal	1625.98 ± 990.46/2038.05 ± 1763.23	0.425
IBD related surgery (Yes/No)	1928.44 ± 1720.74/2011.46 ± 1573.44	0.838
Musculoskeletal EIMs (Yes/No)	1775.10 ± 1487.08/2118.44 ± 1646.59	0.295
Biologics (Yes/No)	2023.79 ± 1631.99/1952.29 ± 1547.90	0.825
Lifetime steroids (Yes/No)	2028.15 ± 1672.12/1844.07 ± 1168.18	0.568
Immunomodulators (Yes/No)	2148.80 ± 1746.68/1653.14 ± 1126.05	0.144
Endoscopic findings (moderate-severe/mild)	2208.27 ± 1836.48/1848.46 ± 1388.93	0.726
HBI (≤4 vs. >4) (for CD)	1978.09 ± 1413.22/1883.1 ± 2180.75	0.846
SCCAI (≤2 vs. >2) (for UC)	2094.3 ± 1668.67/1867.4 ± 1181.45	0.691
CRP (Positive/negative)	2066.74 ± 1540.76/1962.07 ± 1627.53	0.755
Hypertension (yes/no)	1865.08 ± 1568.89/2081.68 ± 1615.16	0.501
Diabetes Mellitus (yes/no)	1530.62 ± 1185.46/2051.37 ± 1630.69	0.308
Statins (yes/no)	1935.65 ± 1630.13/2020.88 ± 1588.25	0.806
Obese (BMI ≥ 30)/non-obese	1748.99 ± 1116.69/2101.31 ± 1752.98	0.304
Ever smoker/never smoker	2021.83 ± 1516.40/1923.47 ± 1823.04	0.784

IBD, Inflammatory bowel disease; SD, standard deviation; UC, ulcerative colitis; CD, Crohn's Disease; HBI, Harvey Bradshaw Index; SCCAI, Simple Clinical Colitis Activity Index; CRP, C-reactive protein; BMI, body mass index.

3.2. Subgroup Analysis

In the group of 79 IBD patients without CVD, extensive UC, immunomodulator use, and the absence of musculoskeletal EIMs were significantly associated with elevated mean SA levels (2539.69 ± 1969.29 pg/mL vs. 1164.45 ± 818.89 pg/mL, $p = 0.044$, 2317.69 ± 1867.99 pg/mL vs. 1451.40 ± 1024.20 pg/mL, $p = 0.043$ and 2340.58 ± 1850.16 pg/mL vs. 1392.09 ± 1054.99 pg/mL, $p = 0.027$, respectively) (Table 3).

Correlation analysis showed a moderate positive correlation between UC extent and SA levels in IBD patients without concomitant CVD comorbidity (r = 0.525, $p = 0.004$) (Table 4). Weak negative correlations between age at study entry, history of musculoskeletal EIMs, and SA levels in the same IBD group were observed (r = −0.263, $p = 0.001$ and r = −0.336, $p = 0.005$, respectively). A weak positive correlation between immunomodulator use and SA levels in IBD patients without CVD was also found (r = 0.26, $p = 0.021$).

In the multivariate analysis of covariance for 79 IBD patients without CVD history (including all significant parameters from univariate analysis and all confounding CVD factors), history of musculoskeletal EIMs was the only factor found to be significantly inversely associated with SA levels ($F(2, 52) = 4.288$, $p = 0.043$), with a small effect size (partial eta squared 0.076).

Subgroup analysis was also performed with a 1:1:2 ratio among twenty-five patients with IBD and concurrent CVD compared to 25 patients with IBD without CVD (age- and sex-matched) and 50 healthy (age- and sex-matched) controls. Both IBD patients with or without CVD had significantly higher SA levels compared to controls (1743.01 ± 1116.26 pg/mL vs. 1283.92 ± 726.85 pg/mL, $p = 0.035$ and 2076.44 ± 1714.74 pg/mL vs. 1525.75 ± 818.74 pg/mL, $p = 0.011$, respectively) (Table 5). No further analysis was conducted due to the small sample size.

Table 3. Subgroup univariate analysis among IBD patients without established CVD and their matched controls.

	Mean Apelin (±SD) (pg/mL)	*p* Value
IBD without CVD (N = 79)**/Controls** (N = 79)	2076.44 ± 1714.74/1525.75 ± 818.74	**0.011**
CD (N = 49)**/UC** (N = 30)	2073.48 ± 1689.54/2081.28 ± 1784.30	0.985
UC E3 (N = 10)**/E1, E2** (N = 20)	2539.69 ± 1969.29/1164.45 ± 818.89	**0.044**
CD B1/CD B2, B3	1953.15 ± 1120.63/2247.96 ± 2303.62	0.554
Biologics Yes (N = 50)**/No** (N = 29)	2097.26 ± 1727.88/2040.55 ± 1721.65	0.888
Steroids lifetime Yes (N = 64)**/No** (N = 15)	2141.36 ± 1821.31/1799.48 ± 1163.26	0.491
Immunomodulators Yes (N = 57)/**No** (N = 22)	2317.69 ± 1867.99/1451.40 ± 1024.20	**0.043**
IBD surgery Yes (N = 15)/**No** (N = 64)	1919.87 ± 1783.49/2113.14 ± 1710.66	0.697
EIMS musculoskeletal Yes (N = 22)/**No** (N = 57)	1392.09 ± 1054.99/2340.58 ± 1850.16	**0.027**
Endoscopic activity Moderate-Severe (N = 32)/Mild (N = 43)	2303.97 ± 1968.41/1947.36 ± 1544.43	0.382
Obese (BMI $\geq$ 30) (N = 22)/**Non-obese** (N = 57)	1768.74 ± 1104.04/2195.21 ± 1893.89	0.325
Ever smoker (N = 55)/**Never smoker** (N = 24)	2127.88 ± 1638.12/1958.58 ± 1910.65	0.689
Hypertension Yes (N = 20)/**No** (N = 59)	1995.25 ± 1899.35/2103.97 ± 1664.16	0.808
Diabetes Mellitus Yes (N = 5)/**No** (N = 74)	1848.78 ± 1631.76/2091.83 ± 1729.75	0.761
Statins Yes (N = 11)/**No** (N = 68)	2246.58 ± 2248.14/2048.92 ± 1631.94	0.725
CRP positive (N = 25)/**CRP negative** (N = 54)	2292.57 ± 1609.17/1976.38 ± 1767.06	0.449

IBD, inflammatory bowel disease; CVD, cardiovascular disease; SD, standard deviation; UC, ulcerative colitis; CD, Crohn's Disease; EIMs, extraintestinal manifestations; BMI, body mass index; CRP, C-reactive protein.

Table 4. Spearman rho correlation between serum apelin levels and IBD-related parameters or CVD risk factors among (a) 104 IBD patients and (b) 79 IBD patients without CVD.

	IBD No CVD (79 pts) (Serum Apelin) Correlation Coefficient(ρ)	*p* Value	ALL IBD (104 pts) (Serum Apelin) Correlation Coefficient(ρ)	*p* Value
CD vs. UC	−0.030	0.795	0.077	0.435
Age at diagnosis	0.136	0.282	0.136	0.282
Disease duration	−0.108	0.395	−0.031	0.787
UC extent E1, E2 vs. E3	**0.525**	**0.004 ***	0.165	0.284
CD Montreal L1 vs. L2, L3	−0.019	0.900	−0.014	0.918
CD Montreal B1 vs. B2, B3	−0.059	0.701	−0.075	0.577
CD Perianal	−0.108	0.507	−0.022	0.872
IMM	**0.26**	**0.021**	0.144	0.144
Biologics	0.063	0.583	0.046	0.64
IBD-related surgery	0.163	0.189	0.085	0.393
Musculoskeletal (EIMs)	**−0.336**	**0.005**	−0.021	0.836
CRP	0.091	0.474	−0.112	0.256
Dyslipidemia	0.009	0.939	−0.101	0.307
Diabetes Mellitus	0.010	0.935	−0.049	0.619
Hypertension	0.096	0.432	−0.051	0.608
Smoking status	−0.085	0.495	−0.042	0.676
BMI	−0.101	0.416	0.094	0.349
Age at study entry	**−0.263**	**0.001**	**−0.214**	**0.002**
Sex (female vs. male)	−0.009	**0.911**	**0.041**	**0.557**

IBD, inflammatory bowel disease; CVD, cardiovascular disease; CD, Crohn's Disease; UC, ulcerative colitis; IMM, immunomodulators; EIMs, extraintestinal manifestations; CRP, C-reactive protein; BMI, body mass index. * Correlation is significant at the level of 0.01.

Table 5. Mean apelin value ± SD differences among IBD patients with CVD and their age and sex matched IBD patients with CVD or the matched control group.

	Mean Apelin (±SD) (pg/mL)	*p* Value
IBD + CVD (N = 25)	1743.01 ± 1116.26	0.122
IBD without CVD (matched) **(N = 25)**	2465.09 ± 2006.51	
IBD + CVD (N = 25)	1743.01 ± 1116.26	**0.035**
Control to IBD + CVD (N = 50)	1283.92 ± 726.85	
IBD without CVD (matched) **(N = 25)**	2465.09 ± 2006.51	**<0.001**
Control to IBD + CVD (N = 50)	1283.92 ± 726.85	

IBD, inflammatory bowel disease; CVD, cardiovascular disease; SD, standard deviation.

4. Discussion

The results of the present study, the first one evaluating SA levels in patients with IBD, showed that IBD patients have higher levels of serum apelin-13 compared to age- and sex-matched healthy controls.

Data on apelin's exact role in intestinal inflammation are scarce. Apelin, like other adipokines, is produced by MAT. In CD, apelin seems to have a regulatory effect on intestinal homeostasis by inducing cell proliferation in the intestinal epithelium and by enhancing lymphatic vessels' function in MAT [21,22]. In models of experimental colitis and human tissues of IBD patients, apelin production has been found to be increased in inflamed colonic tissue, implying a role in the reduction of inflammatory burden and in the healing process in the colon [21]. More recently, Yamada et al. found that apelin levels were downregulated by T-cells rather than colonic epithelial cells in murine models of chronic colitis. When a synthetic apelin peptide was administered, a decrease in proinflammatory cytokines, in combination with improvement in colitis severity and survival rate, was noticed [23]. In line with the above, exogenous apelin administration seems to improve clinical activity and histological inflammation, and enhances colonic tissue repair mechanisms [20,21]. Moreover, exogenous administration of apelin-13 in an experimental rat model of intestinal ischemia/reperfusion showed a positive effect of apelin on oxidative injury at a histopathological level [22]. The finding of increased serum apelin-13 levels in IBD patients in the present study are in accordance with these experimental data. It seems that intestinal inflammation influences apelin production, leading to increased serum apelin levels. These increased apelin levels may contribute to the repair of the intestinal epithelium.

Over the last two decades, there has been growing research on the interaction of visceral adipose tissue (VAT) and the intestinal immune system. Excessive adipose tissue accumulation modulates immune response by activating pro-inflammatory signaling pathways and increasing pro-inflammatory cytokines production, leading to intestinal low-grade inflammation, disruption of intestinal mucosa and permeability, bacterial translocation, and T-cell infiltration, described as "leaky gut". Through this mechanism, excess adipose tissue in terms of obesity has been linked to IBD predisposition [29]. A special form of VAT in CD, known as "creeping fat", is immunologically more active in flares, releasing several cytokines such as TNF α, IL-6, and IL1-β, and adipokines such as leptin, resistin, visfatin, and chemerin. Under these circumstances, creeping fat enhances intestinal inflammation and downregulates other adipokines such as adiponectin, with an inhibitory effect on proinflammatory mediators, promoting further intestinal inflammation [30]. Studies from our laboratory [31] showed increased serum levels of adiponectin, resistin, and ghrelin, and decreased serum levels of leptin in IBD patients. After treatment with infliximab, a significant decrease in serum resistin levels was found [32]. To date, conflicting results exist from a limited number of studies examining associations of serum levels of the aforementioned adipokines with IBD type, clinical, and endoscopic activity [33–37]. The present study revealed that, after the exclusion of patients with CVD, increased SA levels are associated with extensive UC and a history of immunomodulator use ($p = 0.044$ and $p = 0.043$, respectively), both associated with increased inflammatory

burden. These moderate positive correlations suggest a modulating effect of apelin in chronic colonic inflammation.

Regarding treatment, it is known that, in rheumatic diseases such as RA and ankylosing spondylitis, there is no effect of anti-TNF α or disease-modifying antirheumatic drugs on SA levels [12,38]. Similarly, in our study, although we had only a snapshot of the disease, no association of SA and the majority of IBD medications was found; only the immunomodulator use showed a weak association.

In the light of recent studies that have inversely associated apelin plasma levels with the risk of hypertension, the severity of left ventricular dysfunction, the severity of chronic heart failure, and survival in patients with ST-elevation myocardial infarction [11,39–42], we aimed to focus on IBD patients with and without any history of CVD. Although lower in the group of IBD patients with CVD, apelin levels remained significantly higher compared to their matched controls. This could be explained by the impact of IBD systemic inflammation that probably outreaches the CVD lowering effect in SA, as described in studies on EC dysfunction regulatory pathways of apelin in cases with atherosclerosis, coronary artery disease, systemic hypertension, ischemia–reperfusion injury, heart failure, and atrial fibrillation [6].

These findings indicate that CVD history might affect serum apelin levels in patients with IBD, but larger population studies could further elucidate the grade and the significance of these associations.

An interesting finding of our study is that lower mean serum apelin-13 levels were measured in IBD patients with musculoskeletal EIMs compared to IBD patients without. After exclusion of patients with concurrent CVD diagnosis, the history of musculoskeletal EIMs was significantly associated with lower mean apelin levels. It is noticeable that lower serum apelin levels in early rheumatoid arthritis patients compared to healthy controls have been reported [12]. Pathophysiologically, apelin and its receptors have been associated with musculoskeletal system functions including bone formation, skeletal muscle metabolism, and articular inflammation [43]. Our finding may imply that IBD patients with articular EIMs share common pathogenetic pathways with rheumatological diseases. It could be suggested that IBD articular extraintestinal disease enhances inflammatory pathways related to endothelial dysfunction in an analogous way to RA.

Several studies have confirmed the association of apelin with energy metabolism, obesity, and type II diabetes [44–46]. Elevated plasma apelin-12 levels were measured in patients with morbid obesity and correlated positively with BMI [14]. Obesity and high fat diet-induced chronic inflammation has been linked to increased risk of UC and CD [47–49]. Interestingly, in our study although non statistically significant mean SA levels were lower in obese patients (BMI > 30) with IBD compared to non-obese patients (Tables 2 and 3). This result might theoretically be related to other attributable mechanisms of chronic bowel inflammation, IBD treatment effects, or unknown factors.

There are some limitations of our study. Firstly, the sample size of our study population is small. Unfortunately, a few months after initiation of the study, the COVID-19 pandemic emerged. No data collection was undertaken during the first wave of the COVID-19 pandemic. In-hospital visits were limited and home telemanagement and telephone follow-up were implemented. It was therefore difficult to perform blood sampling as patients were not willing to attend in-person visits. The most difficult part of the study was the collection of blood samples from blood donors as the COVID-19 pandemic significantly compromised blood donations. Consequently, this emergency delayed and limited the number of study participants. The delay in blood sample collection further delayed serum human apelin kit measurements, which had a rather short expiry date.

Moreover, we included a high percentage of IBD patients with concurrent diagnosis of CVD, which could also explain the old age of our IBD participants on study entry.

Another disadvantage of our study is that we measured only one isoform of apelin, apelin-13; however, it is the most biologically active and predominant isoform [50,51].

In conclusion, our study documents, for the first-time, increased serum apelin levels in IBD patients. Subgroup analysis failed to demonstrate meaningful associations with disease characteristics. The potential role of serum apelin as a disease marker of IBD and its further associations merit investigation in larger studies.

Author Contributions: All authors contributed to the study conception and design. A.M. has made substantial contributions to the study concept and design, patients' interviews, blood collection, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content. K.K. has participated in study concept and design, performed measurements of serum apelin levels in IBD patients and has participated in interpretation of data. O.K. has participated in blood collection, analysis and interpretation of data and critically revised the manuscript. M.K. has participated in study concept and design, serum apelin levels' measurement in IBD patients, analysis and interpretation of data, critical revision of the manuscript for important intellectual content and statistical analysis, and study supervision. I.E.K. has participated in study concept and design, analysis and interpretation of data, critical revision of the manuscript for important intellectual content, and statistical analysis and study supervision. The first draft of the manuscript was written by A.M. and all authors commented on previous versions of the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by a grant from the Hellenic Society of Gastroenterology (protocol number 7597/10-11-2017).

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of the University Hospital of Heraklion, Crete, Greece, approval number 3075/19-02-2019.

Informed Consent Statement: Informed consent was obtained from all patients involved in the study.

Data Availability Statement: Data files are available after request at Gastroenterology Department, University Hospital of Heraklion (corresponding author Ioannis Koutroubakis).

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Recinella, L.; Orlando, G.; Ferrante, C.; Chiavaroli, A.; Brunetti, L.; Leone, S. Adipokines: New Potential Therapeutic Target for Obesity and Metabolic, Rheumatic, and Cardiovascular Diseases. *Front. Physiol.* **2020**, *11*, 578966. [CrossRef]
2. Yao, H.; He, S. Multi-faceted role of cancer-associated adipocytes in the tumor microenvironment (Review). *Mol. Med. Rep.* **2021**, *24*, 866. [CrossRef]
3. Tatemoto, K.; Hosoya, M.; Habata, Y.; Fujii, R.; Kakegawa, T.; Zou, M.-X.; Kawamata, Y.; Fukusumi, S.; Hinuma, S.; Kitada, C.; et al. Isolation and characterization of a novel endogenous peptide ligand for the human APJ receptor. *Biochem. Biophys. Res. Commun.* **1998**, *251*, 471–476. [CrossRef] [PubMed]
4. Kleinz, M.J.; Davenport, A.P. Immunocytochemical localization of the endogenous vasoactive peptide apelin to human vascular and endocardial endothelial cells. *Regul. Pept.* **2004**, *118*, 119–125. [CrossRef]
5. Kleinz, M.J.; Skepper, J.N.; Davenport, A.P. Immunocytochemical localisation of the apelin receptor, APJ, to human cardiomyocytes, vascular smooth muscle and endothelial cells. *Regul. Pept.* **2005**, *126*, 233–240. [CrossRef]
6. Cheng, J.; Luo, X.; Huang, Z.; Chen, L. Apelin/APJ system: A potential therapeutic target for endothelial dysfunction-related diseases. *J. Cell. Physiol.* **2018**, *234*, 12149–12160. [CrossRef] [PubMed]
7. Antushevich, H.; Wójcik, M. Review: Apelin in disease. *Clin. Chim. Acta* **2018**, *483*, 241–248. [CrossRef]
8. Grinstead, C.; Yoon, S. Apelin, a Circulating Biomarker in Cancer Evaluation: A Systematic Review. *Cancers* **2022**, *14*, 4656. [CrossRef]
9. Kadoglou, N.P.; Lampropoulos, S.; Kapelouzou, A.; Gkontopoulos, A.; Theofilogiannakos, E.K.; Fotiadis, G.; Kottas, G. Serum levels of apelin and ghrelin in patients with acute coronary syndromes and established coronary artery disease—KOZANI STUDY. *Transl. Res.* **2010**, *155*, 238–246. [CrossRef]
10. Gupta, M.D.; Girish, M.; Shah, D.; Rain, M.; Mehta, V.; Tyagi, S.; Trehan, V.; Pasha, Q. Biochemical and genetic role of apelin in essential hypertension and acute coronary syndrome. *Int. J. Cardiol.* **2016**, *223*, 374–378. [CrossRef]
11. Chong, K.S.; Gardner, R.S.; Morton, J.J.; Ashley, E.A.; McDonagh, T.A. Plasma concentrations of the novel peptide apelin are decreased in patients with chronic heart failure. *Eur. J. Heart Fail.* **2006**, *8*, 355–360. [CrossRef] [PubMed]
12. Di Franco, M.; Spinelli, F.R.; Metere, A.; Gerardi, M.C.; Conti, V.; Boccalini, F.; Iannuccelli, C.; Ciciarello, F.; Agati, L.; Valesini, G. Serum levels of asymmetric dimethylarginine and apelin as potential markers of vascular endothelial dysfunction in early rheumatoid arthritis. *Mediat. Inflamm.* **2012**, *2012*, 347268. [CrossRef] [PubMed]

13. Boucher, J.; Masri, B.; Daviaud, D.; Gesta, S.; Guigné, C.; Mazzucotelli, A.; Castan-Laurell, I.; Tack, I.; Knibiehler, B.; Carpéné, C.; et al. Apelin, a newly identified adipokine up-regulated by insulin and obesity. *Endocrinology* **2005**, *146*, 1764–1771. [CrossRef] [PubMed]
14. Heinonen, M.; Purhonen, A.; Miettinen, P.; Pääkkönen, M.; Pirinen, E.; Alhava, E.; Åkerman, K.; Herzig, K. Apelin, orexin-A and leptin plasma levels in morbid obesity and effect of gastric banding. *Regul. Pept.* **2005**, *130*, 7–13. [CrossRef] [PubMed]
15. Habchi, M.; Duvillard, L.; Cottet, V.; Brindisi, M.; Bouillet, B.; Beacco, M.; Crevisy, E.; Buffier, P.; Baillot-Rudoni, S.; Verges, B.; et al. Circulating Apelin is increased in patients with type 1 or type 2 diabetes and is associated with better glycaemic control. *Clin. Endocrinol.* **2014**, *81*, 696–701. [CrossRef]
16. Wysocka, M.B.; Pietraszek-Gremplewicz, K.; Nowak, D. The Role of Apelin in Cardiovascular Diseases, Obesity and Cancer. *Front. Physiol.* **2018**, *9*, 557. [CrossRef] [PubMed]
17. Machura, E.; Ziora, K.; Ziora, D.; Świętochowska, E.; Krakowczyk, H.; Halkiewicz, F.; Kasperska-Zajac, A. Serum apelin-12 level is elevated in schoolchildren with atopic asthma. *Respir. Med.* **2012**, *107*, 196–201. [CrossRef]
18. Kredel, L.I.; Siegmund, B. Adipose-tissue and intestinal inflammation–Visceral obesity and creeping fat. *Front. Immunol.* **2014**, *5*, 112587. [CrossRef]
19. Desreumaux, P.; Ernst, O.; Geboes, K.; Gambiez, L.; Berrebi, D.; Müller-Alouf, H.; Hafraoui, S.; Emilie, D.; Ectors‖, N.; Peuchmaur, M.; et al. Inflammatory alterations in mesenteric adipose tissue in Crohn's disease. *Gastroenterology* **1999**, *117*, 73–81. [CrossRef]
20. Guedj, K.; Abitbol, Y.; Cazals-Hatem, D.; Morvan, M.; Maggiori, L.; Panis, Y.; Bouhnik, Y.; Caligiuri, G.; Corcos, O.; Nicoletti, A. Adipocytes orchestrate the formation of tertiary lymphoid organs in the creeping fat of Crohn's disease affected mesentery. *J. Autoimmun.* **2019**, *103*, 102281. [CrossRef]
21. Ge, Y.; Li, Y.; Chen, Q.; Zhu, W.; Zuo, L.; Guo, Z.; Gong, J.; Cao, L.; Gu, L.; Li, J. Adipokine apelin ameliorates chronic colitis in Il-10−/− mice by promoting intestinal lymphatic functions. *Biochem. Pharmacol.* **2018**, *148*, 202–212. [CrossRef] [PubMed]
22. Han, S.; Wang, G.; Qiu, S.; de la Motte, C.; Wang, H.-Q.; Gomez, G.; Englander, E.W.; Greeley, G.H. Increased colonic apelin production in rodents with experimental colitis and in humans with IBD. *Regul. Pept.* **2007**, *142*, 131–137. [CrossRef] [PubMed]
23. Yamada, D.; Kojima, Y.; Hosoya, A.; Suzuki, M.; Watabe, T.; Inoue, T.; Tsugawa, N.; Asakawa, T.; Yonemoto, Y.; Onizawa, M.; et al. Apelin expression is downregulated in T cells in a murine model of chronic colitis. *Biochem. Biophys. Res. Commun.* **2023**, *647*, 72–79. [CrossRef] [PubMed]
24. Williams, B.; Mancia, G.; Spiering, W.; Agabiti Rosei, E.; Azizi, M.; Burnier, M.; Clement, D.L.; Coca, A.; de Simone, G.; Dominiczak, A.; et al. 2018 ESC/ESH Guidelines for the management of arterial hypertension. *Eur. Heart J.* **2018**, *39*, 3021–3104. [CrossRef] [PubMed]
25. Cosentino, F.; Grant, P.; Aboyans, V.; Bailey, C.J.; Ceriello, A.; Delgado, V. 2019 ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD: The Task Force for diabetes, pre-diabetes, and cardiovascular diseases of the European Society of Cardiology (ESC) and the European Association for the Study of Diabetes (EASD). *Eur. Heart J.* **2020**, *41*, 255–323. [PubMed]
26. Mach, F.; Baigent, C.; Catapano, A.L.; Koskinas, K.C.; Casula, M.; Badimon, L.; Chapman, M.J.; De Backer, G.G.; Delgado, V.; Ference, B.A.; et al. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: Lipid modification to reduce cardiovascular risk: The Task Force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and European Atherosclerosis Society (EAS). *Eur. Heart J.* **2020**, *41*, 111–188. [CrossRef] [PubMed]
27. Harvey, R.F.; Bradshaw, J.M. A simple index of Crohn's-disease activity. *Lancet* **1980**, *1*, 514. [CrossRef] [PubMed]
28. Walmsley, R.S.; Ayres, R.C.S.; Pounder, R.E.; Allan, R.N. A simple clinical colitis activity index. *Gut* **1998**, *43*, 29–32. Available online: http://www.ncbi.nlm.nih.gov/pubmed/9771402 (accessed on 1 January 2023). [CrossRef] [PubMed]
29. Nam, S.Y. Obesity-Related Digestive Diseases and Their Pathophysiology. *Gut Liver* **2017**, *11*, 323–334. [CrossRef]
30. Bilski, J.; Mazur-Bialy, A.; Wojcik, D.; Surmiak, M.; Magierowski, M.; Sliwowski, Z.; Pajdo, R.; Kwiecien, S.; Danielak, A.; Ptak-Belowska, A.; et al. Role of Obesity, Mesenteric Adipose Tissue, and Adipokines in Inflammatory Bowel Diseases. *Biomolecules* **2019**, *9*, 780. [CrossRef]
31. Karmiris, K.; Koutroubakis, I.E.; Xidakis, C.; Polychronaki, M.; Voudouri, T.; Kouroumalis, E.A. Circulating levels of leptin, adiponectin, resistin, and ghrelin in inflammatory bowel disease. *Inflamm. Bowel Dis.* **2006**, *12*, 100–105. [CrossRef] [PubMed]
32. Karmiris, K.; Koutroubakis, I.E.; Xidakis, C.; Polychronaki, M.; Kouroumalis, E.A. The effect of infliximab on circulating levels of leptin, adiponectin and resistin in patients with inflammatory bowel disease. *Eur. J. Gastroenterol. Hepatol.* **2007**, *19*, 789–794. [CrossRef] [PubMed]
33. Weidinger, C.; Ziegler, J.F.; Letizia, M.; Schmidt, F.; Siegmund, B. Adipokines and Their Role in Intestinal Inflammation. *Front. Immunol.* **2018**, *9*, 1974. [CrossRef] [PubMed]
34. Valentini, L.; Wirth, E.K.; Schweizer, U.; Hengstermann, S.; Schaper, L.; Koernicke, T.; Dietz, E.; Norman, K.; Buning, C.; Winklhofer-Roob, B.M.; et al. Circulating adipokines and the protective effects of hyperinsulinemia in inflammatory bowel disease. *Nutrition* **2009**, *25*, 172–181. [CrossRef] [PubMed]
35. Trejo-Vazquez, F.; Garza-Veloz, I.; Villela-Ramirez, G.A.; Ortiz-Castro, Y.; Mauricio-Saucedo, P.; Cardenas-Vargas, E.; Diaz-Baez, M.; Cid-Baez, M.A.; Castañeda-Miranda, R.; Ortiz-Rodriguez, J.M.; et al. Positive association between leptin serum levels and disease activity on endoscopy in inflammatory bowel disease: A case-control study. *Exp. Ther. Med.* **2018**, *15*, 3336–3344. [CrossRef]

36. Konrad, A.; Lehrke, M.; Schachinger, V.; Seibold, F.; Stark, R.; Ochsenkühn, T.; Parhofer, K.G.; Göke, B.; Broedl, U.C. Resistin is an inflammatory marker of inflammatory bowel disease in humans. *Eur. J. Gastroenterol. Hepatol.* **2007**, *19*, 1070–1074. [CrossRef]
37. Tuzun, A.; Uygun, A.; Yesilova, Z.; Ozel, A.M.; Erdil, A.; Yaman, H.; Bagci, S.; Gulsen, M.; Karaeren, N.; Dagalp, K. Leptin levels in the acute stage of ulcerative colitis. *J. Gastroenterol. Hepatol.* **2004**, *19*, 429–432. [CrossRef] [PubMed]
38. Genre, F.; Miranda-Filloy, J.A.; López-Mejias, R.; Carnero-López, B.; Ochoa, R.; Rueda, J.; González-Juanatey, C.; Blanco, R.; Llorca, J.; González-Gay, M.A. Apelin serum levels in non-diabetic ankylosing spondylitis patients undergoing TNF-α antagonist therapy. *Clin. Exp. Rheumatol.* **2013**, *31*, 532–537.
39. Xie, H.; Luo, G.; Zheng, Y.; Hu, D.; Peng, F.; Xie, L. Lowered circulating apelin is significantly associated with an increased risk for hypertension: A meta-analysis. *Clin. Exp. Hypertens.* **2017**, *39*, 435–440. [CrossRef]
40. Tycinska, A.M.; Sobkowicz, B.; Mroczko, B.; Sawicki, R.; Musial, W.J.; Dobrzycki, S.; Waszkiewicz, E.; Knapp, M.A.; Szmitkowski, M. The value of apelin-36 and brain natriuretic peptide measurements in patients with first ST-elevation myocardial infarction. *Clin. Chim. Acta* **2010**, *411*, 2014–2018. [CrossRef]
41. Kuklinska, A.M.; Sobkowicz, B.; Sawicki, R.; Musial, W.J.; Waszkiewicz, E.; Bolinska, S.; Małyszko, J. Apelin: A novel marker for the patients with first ST-elevation myocardial infarction. *Heart Vessel.* **2010**, *25*, 363–367. [CrossRef] [PubMed]
42. Sagiroglu, T.; Oguz, S.; Sagıroglu, G.; Copuroglu, E.; Yalta, T.; Sayhan, M.B.; Yagci, M.A. The effects of apelin on mesenteric ischemia and reperfusion damage in an experimental rat mode. *Balk. Med. J.* **2012**, *29*, 148–152. [CrossRef] [PubMed]
43. Luo, J.; Liu, W.; Feng, F.; Chen, L. Apelin/APJ system: A novel therapeutic target for locomotor system diseases. *Eur. J. Pharmacol.* **2021**, *906*, 174286. [CrossRef] [PubMed]
44. Schinzari, F.; Veneziani, A.; Mores, N.; Barini, A.; Di Daniele, N.; Cardillo, C.; Tesauro, M. Beneficial effects of apelin on vascular function in patients with central obesity. *Hypertension* **2017**, *69*, 942–949. [CrossRef] [PubMed]
45. Bertrand, C.; Pradère, J.-P.; Geoffre, N.; Deleruyelle, S.; Masri, B.; Personnaz, J.; Le Gonidec, S.; Batut, A.; Louche, K.; Moro, C.; et al. Chronic apelin treatment improves hepatic lipid metabolism in obese and insulin-resistant mice by an indirect mechanism. *Endocrine* **2018**, *60*, 112–121. [CrossRef] [PubMed]
46. Li, L.; Yang, G.; Li, Q.; Tang, Y.; Yang, M.; Yang, H.; Li, K. Changes and relations of circulating visfatin, apelin, and resistin levels in normal, impaired glucose tolerance, and type 2 diabetic subjects. *Exp. Clin. Endocrinol. Diabetes* **2006**, *114*, 544–548. [CrossRef] [PubMed]
47. Teixeira, L.G.; Leonel, A.J.; Aguilar, E.C.; Batista, N.V.; Alves, A.C.; Coimbra, C.C.; Ferreira, A.V.; De Faria, A.M.C.; Cara, D.C.; Alvarez Leite, J.I. The combination of high-fat diet-induced obesity and chronic ulcerative colitis reciprocally exacerbates adipose tissue and colon inflammation. *Lipids Health Dis.* **2011**, *10*, 204. [CrossRef] [PubMed]
48. Khalili, H.; Ananthakrishnan, A.N.; Konijeti, G.G.; Higuchi, L.M.; Fuchs, C.S.; Richter, J.M.; Chan, A.T. Measures of obesity and risk of crohn's disease and ulcerative colitis. *Inflamm. Bowel Dis.* **2015**, *21*, 361–368. [CrossRef]
49. Kim, S.-E.; Choo, J.; Yoon, J.; Chu, J.R.; Bae, Y.J.; Lee, S.; Park, T.; Sung, M.-K. Genome-wide analysis identifies colonic genes differentially associated with serum leptin and insulin concentrations in C57BL/6J mice fed a high-fat diet. *PLoS ONE* **2017**, *12*, e0171664. [CrossRef]
50. Chaves-Almagro, C.; Castan-Laurell, I.; Dray, C.; Knauf, C.; Valet, P.; Masri, B. Apelin receptors: From signaling to antidiabetic strategy. *Eur. J. Pharmacol.* **2015**, *763*, 149–159. [CrossRef]
51. Maguire, J.J.; Kleinz, M.J.; Pitkin, S.L.; Davenport, A.P. [Pyr1]apelin-13 identified as the predominant apelin isoform in the human heart: Vasoactive mechanisms and inotropic action in disease. *Hypertension* **2009**, *54*, 598–604. [CrossRef] [PubMed]