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# Adipocytokine plasma concentrations reflect influence of inflammation but not body mass index (BMI) on clinical outcomes of COVID-19 patients: A prospective observational study from the Netherlands

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## Summary

Obesity is recognized as a risk factor for adverse outcome in COVID-19, but the molecular mechanisms underlying this relationship remain unknown. Adipose tissue functions as an endocrine organ by secreting multiple pro-inflammatory and anti-inflammatory factors, known as adipocytokines, which could be involved in COVID-19 severity. We explored the role of adipocytokines in COVID-19 and its association with BMI, clinical outcome, and inflammation. This is an observational study in 195 hospitalized COVID-19 patients. Serial plasma concentrations of the adipocytokines leptin, adiponectin, resistin, and various inflammatory cytokines were assessed. Adipocytokines were compared between patients with normal weight (BMI: 18.5–24.9 kg/m$^2$), overweight (BMI: 25.0–29.9 kg/m$^2$), and obesity (BMI ≥ 30 kg/m$^2$), between patients admitted to the ICU and to non-ICU clinical wards, and between survivors and non-survivors. Patients with overweight and obesity displayed higher leptin concentrations and lower adiponectin concentrations throughout hospital admission ($p < .001$), whereas resistin concentrations were not different from patients with normal weight ($p = .12$). Resistin concentrations correlated with inflammatory markers and were persistently higher in ICU patients and non-survivors compared to non-ICU patients and survivors, respectively (both $p < .001$), whereas no such relationships were found for the other adipocytokines. In conclusion, leptin and adiponectin are associated with BMI, but not with clinical outcomes and inflammation in COVID-19 patients. In contrast, resistin is not associated with BMI, but high concentrations are associated with worse clinical outcomes and more pronounced inflammation. Therefore, it is unlikely that BMI-related adipocytokines or differences in the inflammatory response underlie obesity as a risk factor for severe COVID-19.

KEYWORDS
Adipocytokines, BMI, COVID-19, inflammation, obesity

## 1 | INTRODUCTION

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, has resulted in severe morbidity and mortality worldwide. As of 1 September 2022, the global number of confirmed cases rose above 600 million with more than 6 million deaths. Specifically for the Netherlands, over 8 million confirmed and 20 000 fatal cases were reported, resulting in a cumulative case fatality rate of 0.27%. An increasing number of studies demonstrate an association of obesity with an unfavourable course in COVID-19. Associations between increased body mass index (BMI) and hospitalization,[1–3] as well as with the likelihood to require treatment in the Intensive Care Unit (ICU) have been reported.[3–6] Due to this, overall mortality rates are higher in COVID-19 patients with overweight or obesity,[3,5–7] although once in the ICU, there is no relationship between BMI and clinical outcome.[8–10]

Several potential mechanisms may explain the relationship between obesity and COVID-19. First, angiotensin-converting enzyme 2 (ACE2) expression in adipocytes is even higher compared to that in the lungs, the main tissue affected by COVID-19.[11] Higher expression of the receptor used by SARS-CoV-2 to enter host cells may facilitate viral entry and induce a reservoir for the virus in patients with obesity.[12,13] This might lead to a persisting high viral load and inflammatory response with subsequent adverse outcomes. Second, the adipose tissue functions as an endocrine organ by secreting multiple pro-inflammatory and anti-inflammatory factors, known as adipocytokines. Obesity causes dysregulation of adipocytokine secretion by the adipose tissue which leads to a chronic inflammatory status and an inadequate immune response to infections in patients with obesity.[14] These changes in circulating adipocytokine concentrations could amplify the inflammatory loop, and subsequently, contribute to a hyperinflammatory state in patients with severe COVID-19.

Studies investigating adipocytokines in non-COVID-19-related sepsis have reported increased concentrations of leptin and resistin and decreased concentrations of adiponectin, although results are inconsistent.[15–18] These markers are known to be involved in the inflammatory response in sepsis, with pro-inflammatory effects reported for leptin and resistin, and anti-inflammatory effects for adiponectin.[19] As such, the use of these adipocytokines as diagnostic and prognostic biomarkers in sepsis has been proposed.[19,20]

In the light of obesity being a risk factor for developing severe COVID-19, a more prominent role of adipocytokines in COVID-19 is hypothesized. In previous studies, higher circulating concentrations of leptin[21] and lower concentrations of adiponectin[22] in COVID-19 patients were found compared to critically ill patients without COVID-19. Furthermore, pro-inflammatory effects of adipocytokines are suggested to be in involved in COVID-19,[23–26] but the relation between these mediators, obesity, inflammatory response and COVID-19 severity remains incompletely understood.

The present study aims to elucidate the role of adipocytokines as a potential mechanism underlying obesity as a risk factor for severe COVID-19. We hypothesize that COVID-19 patients with obesity have a more pro-inflammatory phenotype, related to higher concentrations of leptin and resistin, as well as lower concentrations of adiponectin. To explore this, we assessed the kinetics of adipocytokine concentrations in the plasma of COVID-19 patients and investigated associations between these mediators and BMI, clinical outcomes, and inflammation.

## 2 | MATERIALS AND METHODS

### 2.1 | Case definition

In this prospective observational study, all adult patients with a PCR-proven or clinically diagnosed SARS-CoV-2 infection admitted to the Radboud University Medical Center between 6 March and 15 May 2020 were eligible for participation. Clinical diagnosis of COVID-19 was defined based on signs and symptoms, specific computed tomography (CT) findings according to the Dutch COVID-19 Reporting and Data System (CO-RADS) classification,[27] and final consensus of clinical experts. Besides these inclusion criteria, no specific exclusion criteria were applied.

### 2.2 | Data collection

Ethylenediaminetetraacetic acid (EDTA) plasma samples were collected sequentially (every 48–72 hours) during morning routine blood collections for laboratory testing. EDTA plasma and demographic data from a selection of 10 healthy controls, with similar age range compared to the patients in the study, were used for comparison (the 200 Functional Genomics cohort; http://www.humanfunctionalgenomics.org). Clinical data and laboratory results were collected from electronic patient files (EPIC, EPIC Systems) and recorded in electronic Case Report Forms (Castor EDC). The date of hospital admission (or the date of initial hospital admission for patients transferred from another hospital) was recorded. Values of leukocyte counts, leukocyte differentiation, and plasma concentrations of C-reactive protein (CRP) and ferritin were collected at the days of plasma sampling. Clinical outcomes (ICU admission, hospital length of stay, and mortality) were recorded until hospital discharge. Data were aligned for days after initial hospital admission and binned into clusters of 3 days because of variation in the time of sampling. The last observation carried forward

(LOCF) method was used for the imputation of missing timepoints after hospital discharge or death.

### 2.3 | Diagnostic tests

Venous blood was collected in EDTA tubes and subsequently centrifuged at 2954*g* (3800 RPM) at room temperature for 10 min. Plasma was collected and aliquoted before storage at −80 °C for further analysis. Plasma concentrations of leptin, adiponectin, and resistin were measured using enzyme-linked immunosorbent assays (DuoSet ELISA, R&D systems) according to the manufacturer's protocol, with lower detection limits of 0.78 ng/ml, 0.16 μg/ml, and 0.78 ng/ml, respectively. Concentrations of interferon gamma (IFN-γ), interleukin (IL)-10, IL-1 receptor antagonist (IL-1RA), IL-4, IL-6, IL-8, IFN-γ-induced protein (IP)-10, monocyte chemoattractant protein (MCP)-1, macrophage inflammatory protein (MIP)-1α, and tumour necrosis factor (TNF)-α were determined using a Luminex assay (Milliplex, Millipore). The lower detection limit was 3.2 pg/ml for these cytokines.

### 2.4 | Statistical analysis

Data were analysed using SPSS software version 25.0 (IBM) and GraphPad Prism software version 8.0 (GraphPad Software). Differences between groups were assessed by Mann–Whitney *U* tests or Kruskal-Wallis tests with Dunn's multiple comparisons tests for continuous variables and by Fisher's exact tests for categorical variables. Correlations between BMI, adipocytokines, and inflammatory parameters were assessed by Spearman rank correlation tests. Longitudinal data on adipocytokines were analysed by linear mixed-effects model analyses followed by Sidak's post hoc multiple comparison tests on log-transformed data. In case the group*time interaction *p*-value was significant, separate group and time *p*-values are not reported as they are non-interpretable. Binary logistic regression was used to assess the effect of resistin, age, and BMI on mortality. Receiver operating characteristic (ROC) analyses were performed to assess the prognostic performance of resistin by calculating the area under the curve (AUC). The optimal cut-off value was defined based on the maximal Youden's J index and used to assess differences in survival during hospital admission for high versus low resistin concentrations by Kaplan–Meier survival analysis. Hazard ratios were based on the log-rank (Mantel-Cox) test. A *p*-value <.05 (two-tailed) was considered statistically significant. Bonferroni correction for multiple testing was applied where appropriate.

## 3 | RESULTS

### 3.1 | Patient characteristics

A total of 218 patients diagnosed with COVID-19 and admitted to Radboudumc were assessed for study inclusion. Of these, 11 patients refused to participate. Moreover, 12 other patients were excluded because of incomplete clinical data on BMI ($n = 8$) or only available samples after ICU discharge ($n = 4$). Plasma adipocytokine concentrations were assessed in the final study population of 195 hospitalized COVID-19 patients. Ninety-five percent (186/195) of COVID-19-diagnosed patients had a positive PCR at the time of diagnosis. The study population was divided in groups based on BMI (normal weight [BMI: 18.5–24.9 kg/m$^2$, $n = 58$] vs. overweight [BMI: 25.0–29.9 kg/m$^2$, $n = 93$] vs. obesity [BMI ≥ 30 kg/m$^2$, $n = 41$]), ICU admission (non-ICU [$n = 119$] vs. ICU [$n = 70$]), and mortality (survivors [$n = 167$] vs. non-survivors [$n = 28$]). Underweight patients (BMI < 18.4; $n = 3$) were excluded from the BMI analysis. A total of 6 patients were initially admitted to the clinical wards but required ICU care during admission. These patients were excluded from the non-ICU/ICU analyses in order to maintain a dichotomous outcome variable. Table 1 shows higher leukocyte and neutrophil counts, lower lymphocyte counts, and higher concentrations of CRP, ferritin, and D-dimer for ICU patients compared to non-ICU patients. Moreover, ICU patients had a longer hospital stay (33 [20–48] days vs. 7 [7–9] days; $p < .001$), and a higher hospital mortality rate (24% vs. 8%; $p = .004$) compared to non-ICU patients. Non-survivors were older than survivors (73 [69–76] years vs. 64 [53–71] years; $p < .001$), had higher creatinine concentrations, and were more likely to be admitted to the ICU (64% vs. 35%; $p = .006$). No other relevant differences in sex, BMI, and comorbidities were observed between the groups (Table 1). Characteristics of the healthy controls are provided in the Table S1.

### 3.2 | Adipocytokines and obesity in COVID-19 patients

Patients with obesity exhibited higher plasma concentrations of leptin on hospital admission compared to patients with normal weight and overweight ($p < .001$; Figure 1A) and this difference persisted at subsequent timepoints ($p < .001$ for all timepoints), with no between-group differences in kinetics during hospital stay ($p = .92$). Leptin concentration were also higher for patients with overweight compared to patients with a normal weight on all timepoints ($p < .05$ for all timepoints), except baseline. Adiponectin concentrations were higher in patients with normal weight compared to a patient with overweight and obesity on hospital admission ($p < .01$; Figure 1B) and on subsequent timepoints ($p < .05$ for all timepoints). Resistin concentrations were similar between patients with normal weight, overweight, and obesity on hospital admission ($p = .12$; Figure 1C), and did also not change over time in the groups ($p = .17$). BMI was positively correlated with leptin ($r = 0.49$, $p < .001$; Figure S1), whereas an inverse correlation with adiponectin, was observed ($r = -0.27$, $p < .001$; Figure S1). The correlation between BMI and resistin was much weaker ($r = 0.16$, $p = .02$; Figure S1).

### 3.3 | Adipocytokines and clinical outcomes in COVID-19 patients

We observed that BMI was not different between non-ICU and ICU patients with COVID-19 and healthy controls (26.5 [23.8–29.3] kg/

**TABLE 1** Patient characteristics of COVID-19 patients.

| | All patients ($n = 195$) | Normal weight[a] ($n = 58$) | Overweight ($n = 93$) | Obese ($n = 41$) | $p$-value[b] | Non-ICU ($n = 119$) | ICU[c] ($n = 70$) | $p$-value[d] | Survivors ($n = 167$) | Non-survivors ($n = 28$) | $p$-value[e] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Age (years)** | 66 (54–73) | 66 (53–71) | 67 (57–73) | 62 (52–73) | .45 | 67 (53–73) | 64 (57–71) | .25 | 64 (53–71) | 73 (69–76) | **<.001** |
| **Sex, *n* (%)** | | | | | **.04** | | | .51 | | | .83 |
| Male | 135 (69) | 35 (60) | 72 (77) | 25 (61) | | 80 (67) | 51 (73) | | 116 (69) | 19 (68) | |
| Female | 60 (31) | 23 (40) | 21 (23) | 16 (39) | | 39 (33) | 19 (27) | | 51 (31) | 9 (32) | |
| **BMI (kg/m$^2$)** | 26.8 (24.2–29.4) | 23.2 (22.0–24.1) | 27.3 (25.9–28.2) | 32.5 (31.1–35.0) | **<.001** | 26.5 (23.8–29.3) | 27.3 (24.9–29.7) | .36 | 26.9 (24.2–29.6) | 25.9 (24.4–29.0) | .74 |
| **Comorbidity, *n* (%)** | | | | | | | | | | | |
| Diabetes mellitus | 42 (22) | 11 (19) | 20 (22) | 10 (24) | .81 | 25 (21) | 13 (19) | .85 | 35 (21) | 7 (25) | .62 |
| Cardiovascular disease | 107 (55) | 31 (53) | 53 (57) | 22 (54) | .89 | 66 (56) | 37 (53) | .76 | 87 (52) | 20 (71) | .07 |
| Pulmonary disease | 46 (24) | 12 (21) | 17 (18) | 15 (37) | .06 | 34 (29) | 10 (14) | **.03** | 38 (23) | 8 (29) | .48 |
| Malignancy | 52 (27) | 19 (33) | 23 (25) | 9 (22) | .42 | 35 (29) | 15 (21) | .31 | 44 (26) | 8 (29) | .82 |
| **Laboratory values** | | | | | | | | | | | |
| Leukocyte count ($\times 10^9$/L) | 6.6 (5.1–9.7) | 6.0 (4.3–9.1) | 6.6 (5.0–10.1) | 6.9 (5.7–10.3) | .16 | 6.2 (4.2–8.5) | 7.7 (5.8–10.3) | **.003** | 6.5 (5.1–9.7) | 8.2 (4.7–11.0) | .14 |
| Neutrophils ($\times 10^9$/L) | 5.3 (3.5–7.5) | 5.9 (3.5–7.6) | 5.3 (3.5–7.2) | 6.1 (4.2–8.3) | .30 | 4.7 (3.1–6.7) | 6.1 (4.8–8.3) | **.001** | 5.2 (3.3–7.0) | 6.3 (4.0–8.8) | .09 |
| Lymphocytes ($\times 10^9$/L) | 0.7 (0.4–1.1) | 0.6 (0.4–1.0) | 0.7 (0.5–1.1) | 0.8 (0.6–1.2) | .12 | 0.8 (0.5–1.1) | 0.7 (0.4–0.9) | **.03** | 0.7 (0.5–1.1) | 0.5 (0.3–1.2) | .20 |
| CRP (mg/L) | 113 (61–175) | 100 (61–176) | 120 (78–200) | 113 (49–173) | .42 | 83 (46–121) | 194 (139–302) | **<.001** | 113 (58–174) | 138 (80–241) | .21 |
| Ferritin (μg/L) | 1089 (546–1874) | 867 (514–1562) | 1002 (440–2189) | 1345 (860–2193) | .10 | 868 (399–1485) | 1682 (1013–2460) | **<.001** | 997 (489–1874) | 1270 (763–1886) | .19 |
| D-dimer (ng/ml) | 1680 (900–3120) | 1725 (1020–3200) | 1975 (1080–3565) | 1610 (1085–2490) | .75 | 1280 (760–1975) | 3420 (1840–5630) | **<.001** | 1610 (880–2790) | 2020 (1240–3750) | .22 |
| Creatinine (μmol/L) | 81 (65–100) | 87 (68–110) | 88 (76–110) | 88 (69–107) | .27 | 81 (66–96) | 77 (60–114) | .75 | 78 (63–95) | 103 (78–136) | **.002** |
| **ICU admission during a hospital stay, *n* (%)** | 76 (39) | 21 (36) | 38 (41) | 17 (42) | .82 | NA | NA | NA | 58 (35) | 18 (64) | **.006** |
| **Hospital length of stay (days)** | 9 (6–27) | 11 (6–26) | 10 (5–26) | 9 (7–34) | .93 | 7 (7–9) | 33 (20–48) | **<.001** | 9 (6–27) | 17 (6–28) | .29 |
| **Mortality, *n* (%)** | 28 (14) | 9 (16) | 14 (15) | 5 (12) | .89 | 10 (8) | 17 (24) | **.004** | NA | NA | NA |

*Note*: The bold values represent the significance as they are $p$-values. Data are presented as median (IQR) or $n$ (%). Normal weight was defined as BMI: 18.5–24.9 kg/m$^2$, overweight was defined as BMI: 25.0–29.9 kg/m$^2$, and obesity was defined as BMI ≥ 30 kg/m$^2$.

Abbreviations: BMI, body mass index; COVID-19, coronavirus disease 2019; CRP, C-reactive protein; ICU, intensive care unit; NA, not applicable.

[a]Data from 3 patients (2%) with underweight are not shown separately.

[b]Normal weight versus overweight versus obese by Kruskal-Wallis tests.

[c]Data from 6 patients (3%) that were initially admitted to the clinical ward and transferred to ICU during hospital admission are not shown separately.

[d]Non-ICU versus ICU by Mann–Whitney $U$ tests.

[e]Survivors versus non-survivors by Mann–Whitney $U$ tests.

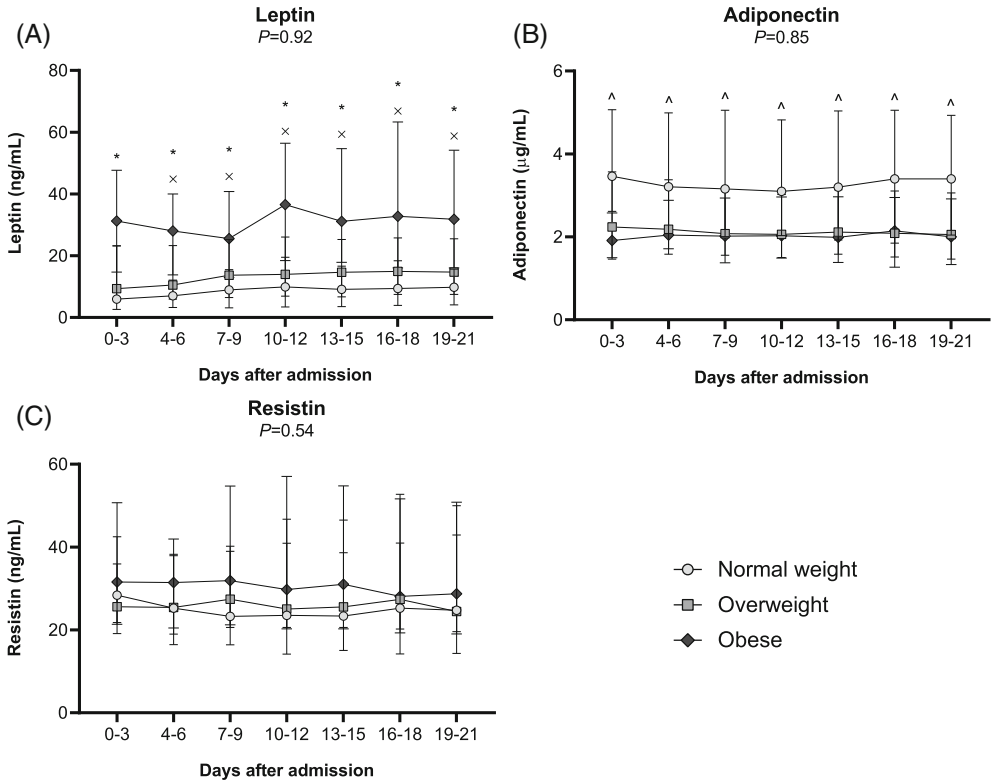


**FIGURE 1** Concentrations of adipocytokines during hospital admission in COVID-19 patients with normal weight, overweight, and obesity. Kinetics of (A) leptin, (B) adiponectin, and (C) resistin plasma concentration patients with COVID-19 with a normal weight ($n = 58$), overweight ($n = 93$), and obesity ($n = 41$). Normal weight was defined as BMI: 18.5–24.9 kg/m$^2$, overweight was defined as BMI: 25.0–29.9 kg/m$^2$, and obesity was defined as BMI ≥ 30 kg/m$^2$. Data are presented as median with interquartile range. Linear mixed-effect models yielded *p*-values for the group*time interaction factor of .92, .85, and .54, for group-factor of <.001, <.001, and .12, and for time factor of <.001, .97, and .17, for leptin, adiponectin, and resistin, respectively. *$p < .05$ between obese versus normal weight and overweight at individual timepoints calculated using Sidak's post-hoc test. ˣ $p < .05$ between overweight versus normal weight at individual timepoints calculated using Sidak's post-hoc test. ^$p < .05$ between normal weight versus overweight and obesity at individual timepoints calculated using Sidak's post-hoc test. The *p*-values in the panels represent the group*time interaction factors. Abbreviations: BMI, body mass index; COVID-19, coronavirus disease 2019.

m$^2$ vs. 27.3 [24.9–29.7] kg/m$^2$ vs. 26.9 [23.5–29.4] kg/m$^2$, respectively; $p = .65$). On hospital admission, circulating leptin and adiponectin concentrations in both ICU and non-ICU COVID-19 patients were similar to those in healthy controls ($p > .99$ for all comparisons; Figure 2A,B), whereas resistin concentrations in both ICU and non-ICU patients were higher compared to healthy controls ($p < .001$ and $p = .04$, respectively; Figure 2C). Between ICU and non-ICU patients, no differences in leptin and adiponectin concentrations on admission were observed ($p = .89$ and $p > .99$, respectively), but resistin concentrations were significantly higher in ICU patients compared to non-ICU patients on hospital admission ($p = .03$). The kinetics of leptin and adiponectin concentrations during hospital stay differed between ICU and non-ICU patients (both $p < .001$; Figure 2A,B), with an increase in leptin concentrations and a slight decrease in adiponectin concentrations in ICU patients. The concentrations of resistin were persistently higher in ICU patients compared to non-ICU patients and no changes in resistin concentrations were observed during hospital admission (Figure 2C).

When comparing surviving and non-surviving COVID-19 patients, we observed that BMI was similar between groups (26.9 [24.2–29.6] kg/m$^2$ vs. 25.9 [24.4–29.0] kg/m$^2$; $p = .75$). Also, the concentrations of circulating leptin, adiponectin, and resistin at the moment of hospital admission did not differ between survivors and non-survivors ($p = .98$, $p = .17$, $p = .56$, respectively; Figure 3). Leptin concentrations increased during hospital stay in both survivors and non-survivors ($p = .01$), with no between-group differences over time ($p = .63$; Figure 3A). Adiponectin did not change over time ($p = .93$), with no differences between groups either ($p = .16$; Figure 3B). Resistin concentrations significantly increased after admission only in non-survivors ($p < .001$; Figure 3C).

The relationship between resistin and mortality was further analysed by binary logistic regression, using the concentration of resistin measured in the sample collected at days 0–3 and 4–6 days after hospital admission. The data listed in Table 2 demonstrate that higher resistin concentrations 4–6 days after hospital admission are associated with an increased risk of death (OR = 1.32 per 10 ng/ml [95%CI 1.10–1.58]; $p = .003$), independent of age and BMI. Furthermore, ROC analyses revealed an AUC for resistin at 4–6 days of 0.76 (95%CI 0.64–0.87) to predict mortality (Figure 4A). Based on the optimal cut-off value, we observed that patients with high concentrations of resistin at days 4–6 of hospital admission had an increased risk for in-hospital mortality (HR = 5.1 [95%CI 2.1–12.1]; $p = .003$; Figure 4B).

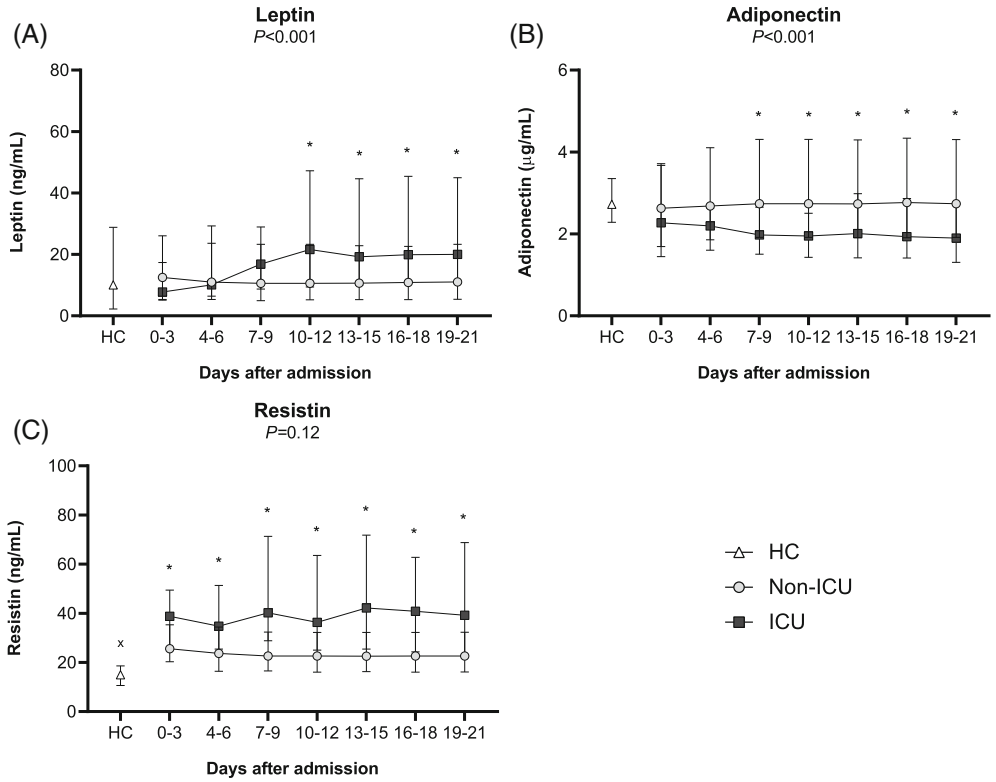


**FIGURE 2** Concentrations of adipocytokines during hospital admission in non-ICU and ICU COVID-19 patients compared to healthy controls. Longitudinal course of (A) leptin, (B) adiponectin, and (C) resistin plasma concentration in non-ICU ($n = 119$) and ICU patients ($n = 70$) with COVID-19 compared to healthy controls ($n = 10$). Data are presented as median with interquartile range. Linear mixed-effect models yielded $p$-values for the group*time interaction factor (non-ICU vs. ICU) of <.001, <.001, .12 for leptin, adiponectin, and resistin, respectively. For resistin, $p$-values of <.001 and .21 were observed for the group-factor and time-factor, respectively. $^{x}$ $p < .05$ for HC compared with non-ICU and ICU patients calculated using Sidak's post-hoc tests; * $p < .05$ between groups at individual timepoints calculated using Sidak's post-hoc tests. The $p$-values in the panels represent the group*time interaction factors. Abbreviations: BMI, body mass index; COVID-19, coronavirus disease 2019; HC, healthy controls; ICU, intensive care unit.

### 3.4 | Correlation of adipocytokines with inflammatory markers and BMI

Next, we explored associations between adipocytokines and markers of inflammation. As depicted in Figure 4C, significant positive correlations between plasma resistin concentrations and classical inflammatory parameters leukocytes and neutrophils, and CRP were observed ($r = 0.38$, corrected $p < .05$, $r = 0.42$, corrected $p < .05$, and $r = 0.36$, corrected $p < .05$, respectively). Similarly, significant positive correlations were observed between plasma concentrations of resistin and circulating levels of inflammatory cytokines IL-10, IL-1RA, IL-6, IL-8, and TNF-α ($r = 0.29$, $r = 0.47$, $r = 0.28$, $r = 0.27$ and $r = 0.42$, respectively; all corrected $p < .05$), indicating an association of resistin with the systemic inflammatory response observed in COVID-19. No significant correlations with inflammatory markers were present for BMI and leptin, whereas adiponectin correlated negatively with leukocyte counts ($r = -0.24$, corrected $p < .05$).

## 4 | DISCUSSION

The adipose tissue is an organ with important endocrine function, and the secretion of metabolic and inflammatory mediators, such as the family of adipocytokines, has important physiological functions. Dysregulation of adipocytokine production can contribute to inflammation in disease processes. A hyperinflammatory reaction is known to contribute to the severity and poor outcome in COVID-19,[28,29] and individuals with overweight and obesity have an increased risk for severe disease and complications.[3,4] Whether the release of inflammatory adipocytokines from the adipose tissue constitutes a link between obesity and severe COVID-19 had not been thoroughly explored. In this study, the dysregulated adipocytokine concentrations in COVID-19 patients with obesity was not related to COVID-19-related mortality. We observed that BMI and plasma concentrations of the BMI-associated adipocytokines leptin and adiponectin are not increased in COVID-19 patients and show no relationship with clinical outcomes or inflammatory markers. In contrast, resistin plasma

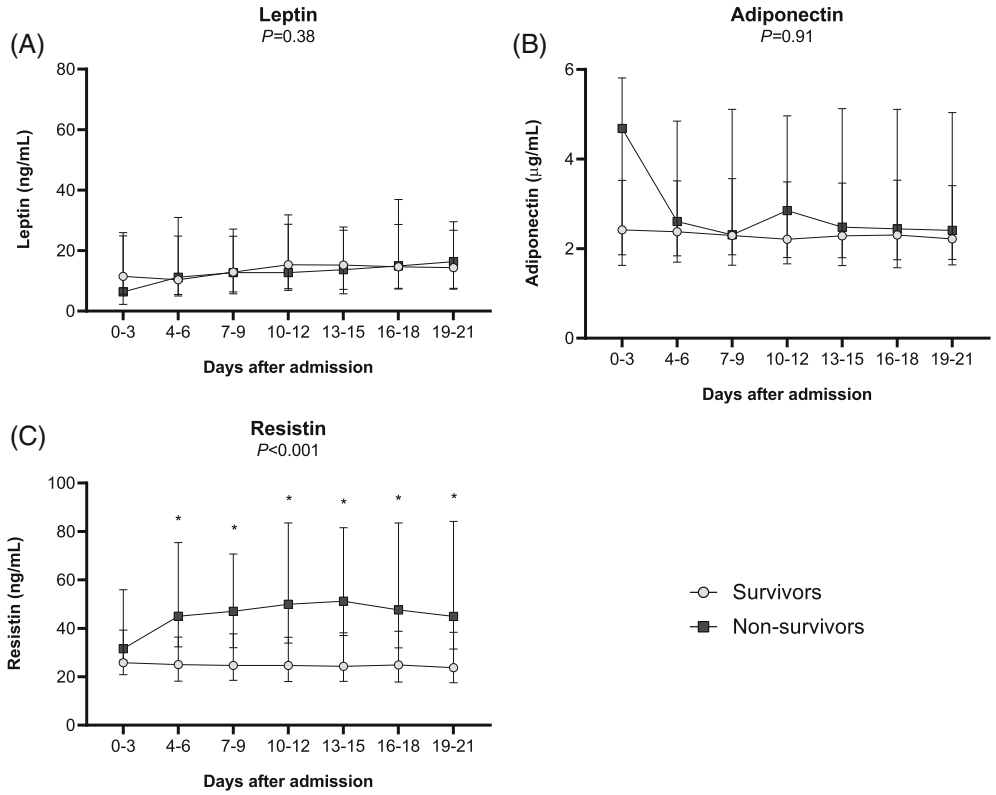


**FIGURE 3** Concentrations of adipocytokines during hospital admission in surviving and non-surviving COVID-19 patients. Longitudinal course of (A) leptin, (B) adiponectin, and (C) resistin plasma concentration in surviving ($n = 167$) and non-surviving ($n = 28$) COVID-19 patients. Data are presented as median with interquartile range. Linear mixed-effect models yielded *p*-values for the group*time interaction factor of .38, .91, <.001 for leptin, adiponectin and resistin, respectively. *p*-values for the group-factor of .63, .16 and for time-factor of .01, .93 were observed for leptin and adiponectin, respectively. * $p < .05$ between groups at individual timepoints calculated using Sidak's post-hoc tests. The *p*-values in the panels represent the group*time interaction factors. Abbreviations: BMI, body mass index; COVID-19, coronavirus disease 2019.

**TABLE 2** Hospital mortality related risk factors in COVID-19 patients.

| | Unadjusted OR | 95%CI | *p*-value | Adjusted OR | 95%CI | *p*-value |
|---|---|---|---|---|---|---|
| **Age (years)** | 1.10 | 1.05–1.15 | **<.001** | 1.12 | 1.03–1.22 | **.008** |
| **BMI (kg/m$^2$)** | 0.98 | 0.90–1.08 | .72 | 0.85 | 0.69–1.05 | .13 |
| **Resistin 0–3 days (per 10 ng/ml)**[a] | 1.30 | 0.97–1.74 | .08 | 1.29 | 0.89–1.88 | .18 |
| **Resistin 4–6 days (per 10 ng/ml)**[b] | 1.29 | 1.09–1.52 | **.003** | 1.32 | 1.10–1.58 | **.003** |

*Note*: The bold values represent the significance as they are *p*-values. Odds ratios were calculated using binary logistic regression.
Abbreviations: BMI, body mass index; OR, odds ratio; 95%CI, 95% confidence interval.
[a]$n = 120$.
[b]$n = 161$.

concentrations are increased in COVID-19 independent of BMI, and are associated with ICU admission, mortality, and inflammation. These findings indicate that differences in BMI do not explain the variability in inflammatory response through differences in adipocytokines. This might be explained by an overriding effect of the acute and profound pro-inflammatory response in severe COVID-19 on the chronic low-grade pro-inflammatory effects of obesity.

As well-established in the literature, this study also demonstrates a positive correlation of leptin and a negative correlation of adiponectin with BMI.[14] However, we observed that these adipocytokines were not associated with clinical outcomes and the inflammatory response in COVID-19 patients. This is similar to the findings of a recent small study that showed no significant correlations of leptin and adiponectin plasma concentrations with inflammatory cytokines, severity and duration of mechanical ventilation in 27 COVID-19 and 36 non-COVID-19 patients.[30]

Higher circulating concentrations of leptin in 31 COVID-19 patients compared to 8 SARS-CoV-2-negative patients have been

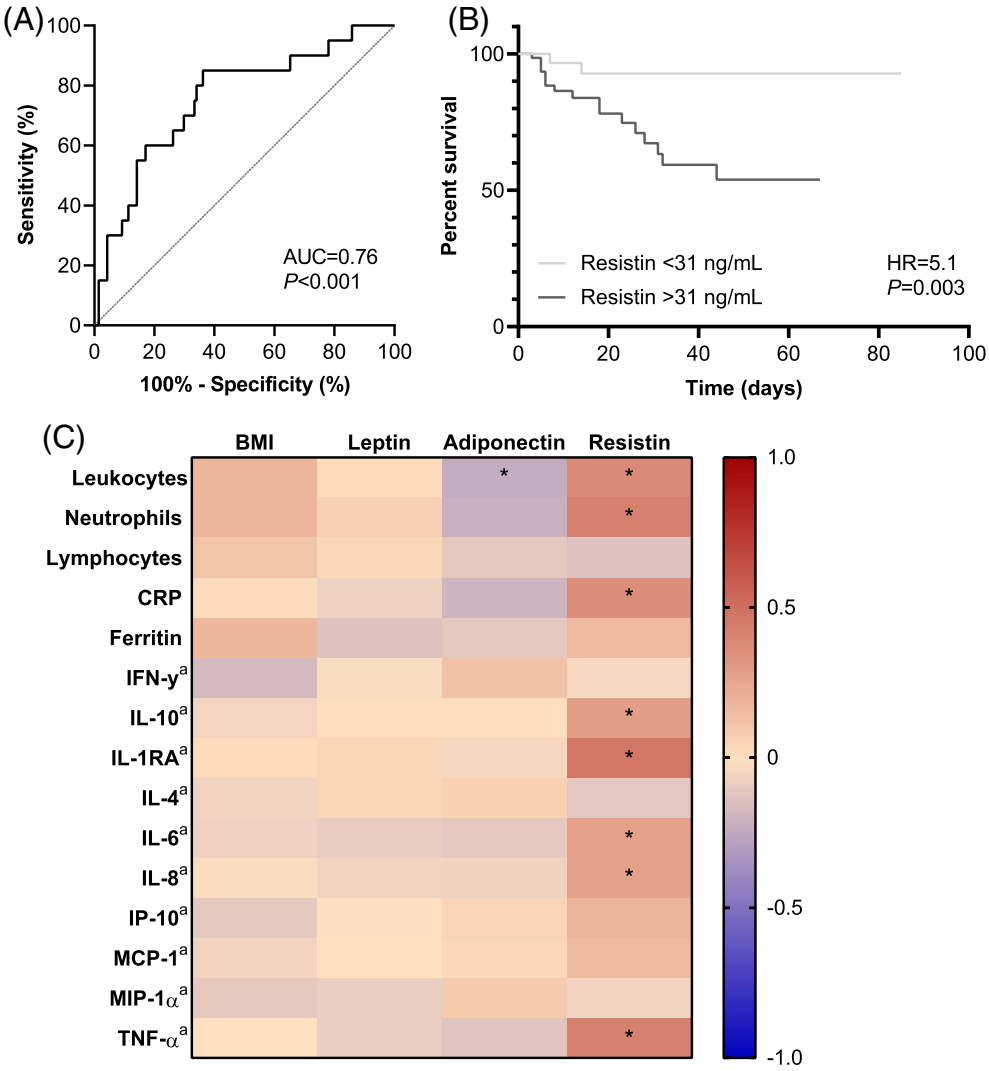


**FIGURE 4** Discriminatory power of resistin and correlations with inflammatory parameters. (A) ROC curve based on mortality for resistin concentrations measured at 4–6 days of hospital admission. (B) Survival analyses for high versus low concentrations of resistin. The optimal cut-off value was based on the maximal Youden's J index derived from the ROC curve. The hazard ratio was calculated with the log-rank (Mantel-Cox) test. (C) Heatmap of the correlations of BMI and adipocytokine concentrations with inflammatory parameters in COVID-19 patients at the first available timepoint. Correlation coefficients and *p*-values were calculated with Spearman rank correlation tests. * $p < .05$ after Bonferroni correction for multiple testing. [a] measurement in 132 of the 195 patients. Abbreviations: COVID-19, coronavirus disease 2019; ROC, receiver-operating characteristic; AUC, area under the curve; HR, hazard ratio; BMI, body mass index; CRP, C-reactive protein; IFN-γ, interferon gamma, IL, interleukin; IL-1RA, interleukin 1 receptor antagonist, IP-10; interferon gamma induced protein; MCP-1, monocyte chemoattractant protein 1; MIP-1α, macrophage inflammatory protein 1 alpha; TNF-α, tumour necrosis factor alpha.

reported in a cross-sectional study measuring leptin concentrations in ICU patients on 1 day in the first wave of the COVID-19 pandemic in the Netherlands.[21] Our study showed no difference in leptin concentrations between COVID-19 patients and healthy controls during the first week of hospital admission. From week 2 onwards, however, higher leptin concentrations were observed in ICU patients compared with non-ICU patients and healthy controls. This indicates that the sampling timepoint is an important factor for leptin plasma measurements and this may explain inconsistent results as were also observed in studies addressing leptin concentrations in sepsis patients. One study described significantly higher serum leptin concentrations in septic patients compared to non-septic patients admitted to the ICU,[15] whereas another study did not observe differences between ICU patients with and without sepsis.[17] Clearly, apart from the impact of variation in BMI, these discrepancies could be due to differences in the time of sampling.

Our study indicates that the role of resistin in COVID-19 differs from that of leptin and adiponectin. Monocytes and macrophages in the adipose tissue are the main source of resistin in humans and resistin has potent pro-inflammatory properties.[19,31] In vitro studies have indicated that resistin leads to an increase in pro-inflammatory cytokines (e.g., IL-6, TNF-α) via induction of the NF-κB pathway.[31–33] Research in sepsis patients suggested a link between resistin and inflammation based on the increased serum resistin concentrations

observed in sepsis patients and the correlation of resistin with inflammatory parameters (CRP, IL-6, TNF-α).[16] In view of the observed correlation between resistin and measured cytokines, our study strengthens the concept of an association between resistin and the systemic inflammatory response in severe infectious diseases, in this case in COVID-19. Next, resistin was proposed as a prognostic biomarker in sepsis, most relevant in combination with other prognostic parameters,[34] and a similar approach could be of value for COVID-19.

This study has several limitations, mostly due to the pragmatic design of this study during the first months of the COVID-19 pandemic. First of all, the observational nature of this study precludes the identification of cause-effect relationships. Next, variation in sampling timepoints between patients was present because blood withdrawals for study purposes were combined with clinical blood collections and because of the high number of transfers from other hospitals. However, since we aligned our data according to the day of the first hospitalization, this has not relevantly influenced the results. A comprehensive longitudinal analysis of adipocytokines and worsening disease course was complicated by the low number of patients transferred from the ward to the ICU during their stay in our hospital. Therefore, no conclusions regarding the direct relationship between adipocytokines and disease deterioration can be drawn from our data. Next, this study presents data collected before the general introduction of immunomodulatory treatments in COVID-19 patients, such as dexamethasone and tocilizumab. Therefore, potential interactions of these drugs with adipocytokines have not been taken into account.[35,36] Also, in contrast to previous findings, we found that BMI was not related to ICU admission and mortality in this study. While a relatively limited statistical power to confirm this relation may also be at play, this gave us the opportunity to study the impact of adipocytokines independently of BMI differences between patient subgroups.

From this study, we can conclude that inflammation rather than obesity is related to disease severity in COVID-19 and that alterations in adipocytokine concentrations do not represent the underlying mechanism for the negative impact of obesity on COVID-19 outcomes. Of interest, independent of BMI, resistin plays a more prominent role in COVID-19, as it is associated with both inflammation and clinical outcome and may therefore hold promise as a prognostic factor.

## AUTHOR CONTRIBUTIONS

Aline H. de Nooijer, Inge Grondman, Emma J. Kooistra, Matthijs Kox, Peter Pickkers, and Mihai G. Netea designed the study. Aline H. de Nooijer, Inge Grondman and Nico A.F. Janssen were responsible for data and sample collection and laboratory processing. Emma J. Kooistra, Matthijs Kox and Peter Pickkers were responsible for sample and data collection of the ICU patients. Aline H. de Nooijer, Emma J. Kooistra, Matthijs Kox, Peter Pickkers and Mihai G. Netea contributed to the data analysis and interpretation. Aline H. de Nooijer drafted the manuscript. Emma J. Kooistra, Inge Grondman, Nico A.F. Janssen, Leo A.B. Joosten, Frank L. van de Veerdonk, Matthijs Kox, Peter Pickkers, and Mihai G. Netea critically revised the manuscript. All authors read and approved the final manuscript.

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## CONFLICT OF INTEREST

Mihai G. Netea is a scientific founder of TTxD. The other authors have no conflict of interest regarding this study.

## ETHICS STATEMENT

The study was carried out in the Netherlands in accordance with the applicable rules concerning the review of research ethics committees and informed consent. The study protocol was approved by the local ethics committee, CMO region Arnhem-Nijmegen, (CMO 2020 6344 and CMO 2016 2963) and performed in accordance with the latest version of the declaration of Helsinki and guidelines for good clinical practice (GCP). All patients or legal representatives were informed about the study details and could decline to participate.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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