The combined effect of adiponectin and resistin on all-cause mortality in patients with type 2 diabetes: Evidence of synergism with abdominal adiposity

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Abstract

Background and aims

While elevated serum adiponectin and resistin levels have been singly associated with all-cause mortality in patients with type 2 diabetes (T2D), their combined effect has never been studied.

We investigated such joint effect in patients with T2D and its possible modulation by several demographic and clinical conditions, known to affect *per se* mortality rate.

Methods

Patients with T2D from the Gargano Mortality Study (GMS; N = 895, follow-up = 10.5 ± 3.7 years; 290 events) and the Foggia Mortality Study (FMS; N = 519, follow-up = 7.1 ± 2.5 years; 140 events) were examined.

Results

As singly considered, adiponectin and resistin were independently associated with mortality rate in GMS and FMS ($p < 0.0001$ for both). The two studies were then pooled, for investigating the nature of the joint effect of the two adipokines. In such sample, both adipokines were associated with death, independent of each other and of several additional covariates ($p = 0.01–4.58 \times 10^{-12}$). Of note, no adiponectin-by-resistin interaction was observed ($p = 0.40$), thus pointing to an additive effect of the two adipokines. As compared to individuals with low levels of both adiponectin and resistin (i.e. below

median values), those with high levels of both adipokines had an HR (95%CI) for death of 3.02 (2.26–4.03). Such increased risk was more pronounced in individuals with relatively low abdominal adiposity (p for HR heterogeneity below or above the median value of waist circumference = 0.03).

Conclusions

Adiponectin and resistin show an additive independent effect on all-cause mortality in patients with T2D. Such effect is modified by abdominal adiposity.

Keywords: Adiponectin; Resistin; Additive effect; Mortality risk; Waist circumference

1 Introduction

Type 2 diabetes (T2D) is a leading risk factor for all-cause death, with mortality rate in diabetic patients being twice as much as that in non-diabetic individuals [1]. Great efforts are, therefore, needed t decrease such tremendous burden. A deeper understanding of the role of biomarkers able to predict mortality rate in T2D may help accomplish this goal.

The last decade has witnessed that some adipokines exert an important role in shaping mortality risk [2–6]; among these, are resistin and adiponectin, known to affect intermediate metabolism [7], low-grad inflammation and atherosclerotic processes [8–10]. Resistin is positively related with all-cause mortality in several sets [6,11], including T2D [6,12]. Quite unexpectedly, a similar positive association across severa conditions [3,4,13–18], comprising T2D [19,20], has been paradoxically reported also for adiponectin. Despite so many data on each of the two adipokines, their combined effect on mortality rate has been investigate only once in a small study, carried out in the specific subset of dialysis patients [21].

Our aim was to explore the interwoven effect of adiponectin and resistin on mortality rate in patients with T2D. In addition, the role of several possible modifiers on such joint effect was investigated.

To pursue this goal, data from over 1,400 Italian diabetic patients followed over time for several years were analyzed.

2 Materials and methods

2.1 The Gargano Mortality Study (GMS)

This cohort comprises 1,028 patients with T2D (according to ADA 2003 indications) consecutively enrolled from November 1st, 2000 to September 30th, 2005 at the Endocrine Unit of IRCCS ''Casa Sollievo della Sofferenza'' i San Giovanni Rotondo (Apulia, central-southern Italy), in a study on all-cause mortality [12,22–24]. Poor life expectancy, due to non diabetes-related disorders, was the only exclusion criterion. GMS has been followed-up until 31s December, 2014. Vital status was ascertained by telephone interview with patients or their relatives. Alternatively, needed information was obtained through the registry office of residence cities. The last follow-up was carried out b inquiries to the Italian Health Card (http://sistemats1.sanita.finanze.it/wps/portal/portalets/cittadinots/ts).

Serum total adiponectin and resistin were assessed in 895 participants (87.1%) constituting the eligible sample for the present analysis.

2.2 The Foggia Mortality Study (FMS)

This cohort comprises 1,253 patients with T2D (ADA 2003) consecutively recruited at the Endocrine Unit of the University of Foggia (Apulia, central-southern Italy) from 7th January, 2002 to 30th September, 2008 for a stud whose end-point was all-cause mortality [23,25].

Also in this case, poor life expectancy due to malignancies was the only exclusion criterion. FMS was followed until 31st March, 2015. At follow-up, vital status of study patients was ascertained by telephone interviews or querie to the registry office of residence cities.

Serum total adiponectin and resistin were measured in 519 patients (45.0%), who constituted the suitable sample for the current analysis.

2.3 Examination at baseline

In both studies, clinical data were obtained at baseline from a standardized interview and examination and blood samples were collected in the morning after an overnight fast. Serum aliquots were stored at −80 °C.

Smoking habits, anti-hypertensive, anti-dyslipidemic, and glucose-lowering treatments were registered at the time of examination. No thiazolidinediones were ever used in these patients. Medical registers confirmed dat concerning medications. Current smokers were patients smoking cigarettes habitually during the year before the examination. Diabetes duration was calculated subtracting from the current age the age at diabetes diagnosis.

Urinary albumin and creatinine measurements at baseline were determined as previously described [26].

The CKD-EPI formula was used in order to estimate eGFR [27].

2.4 Ethics

Institutional Ethic Committee of Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) ''Casa Sollievo della Sofferenza'' and the University of Foggia, respectively authorized both study protocols and informed consen procedures. All participants gave a written informed consent.

2.5 Measurement of circulating adiponectin and resistin levels

Adiponectin and resistin serum concentrations were measured at the Research Unit of Diabetes and Endocrine Diseases at ''Casa Sollievo della Sofferenza'' by commercial ELISA kits (Alpco, Salem, NH and Bio Vendor, Brn Czech Republic respectively), as previously described [28,29]. Adiponectin and resistin inter- and intra-assay coefficients of variation were 7.0 and 6.6%, and 7.0% and 5.2%, respectively.

2.6 Statistical methods

Baseline characteristics of patients are shown as mean ± SD and percentages for continuous and categorical variables, respectively.

The relationship between either adiponectin or resistin serum concentrations and all-cause mortality was log linear, as assessed by the Kolmogorov-type supremum test based on a sample of 10,000 simulated residual pattern [30]. Consequently, both adiponectin and resistin were analyzed after natural log transformation.

In both GMS and FMS, the time variable was defined as the time between the baseline examination and date of death or of the last clinical follow-up for survivors. Incidence rates (IR) for all-cause death were reported as th number of new events per 100 person years (py). The association between adiponectin or/and resistin and the event occurrence was assessed by univariate and multivariable Cox proportional hazards regressions analyses. Risks wer reported as HRs along with their 95% CI for SD increase in natural logarithm of adiponectin or/and resistin levels.

Pooled analyses were performed in an~~a~~ individual data meta-analysis fashion [31], adjusting for "study sample", after testing for heterogeneity (i.e. the presence or absence of a significant exposure-by-sample interaction).

We evaluated six separate models, including several covariates possibly related to the exposures and/or the outcome of our study design. The first model was adjusted only for "study sample" (i.e. GMS and FMS); in the secon model sex, age at recruitment and smoking habits were added to the previous model; in the third model BMI and waist circumference were added to the second model; in the fourth model disease duration and HbA1c and age at diabete diagnosis were added to the third model (in this case, because of its collinearity with diabetes duration, age at recruitment was not included and was replaced by age at diagnosis; in this way the sum of the effect provided by both age a diagnosis and diabetes duration, mathematically correspond to the overall effect provided by the age at recruitment itself); in the fifth model ACR and eGFR were added to the third model; finally, in the sixth model glucose-lowering anti-hypertensive and anti-dyslipidemic treatments were added to the third model.

Lastly, as possible modifiers of the joint effect of adiponectin and resistin on mortality rate, age at recruitment, sex, smoking habits, BMI, waist circumference, HbA1c, diabetes duration, ACR, eGFR and medications, wer investigated. This was tested by Cox proportional hazards analyses stratified according to the above-mentioned variables along with the presence of multiplicative interaction terms.

We considered as statistically significant a p-value <0.05. SPSS v.15 (SPSS, Chicago IL) and SAS Release 9.3 (SAS Institute, Cary, NC) software were utilized for the analyses.

3 Results

Clinical features of patients from GMS and FMS are reported in Table 1. The two samples showed some differences in terms of most clinical variables but, sex, smoking habits, and glucose-lowering treatment Also resistin, but not adiponectin, serum levels were different across the two studies.

Table 1 Clinical characteristics of patients from GMS and FMS.

	GMS (n = 895)	FMS (n = 519)
Males (%)	51.6	50.9
Age at recruitment (yrs)	61.9 ± 9.6	63.2 ± 11.6

Smokers (%)	14.4	15.6
BMI (kg/m^2)	30.9 ± 5.6	29.9 ± 6.1
Waist circumference (cm)	102.3 ± 13.5	105.7 ± 14.7
Diabetes duration (yrs)	10.7 ± 8.9	13.0 ± 10.1
HbA1c (%)	8.7 ± 2.0	9.0 ± 2.2
Glucose-lowering TX (%)	83.2	79.8
Diet only (%)	13.6	11.9
Oral agents	42.1	45.9
Insulin w/wo oral agents (%)	41.1	33.9
Anti-hypertensive TX (%)	51.4	65.3
Anti-dyslipidemic TX (%)	32.4	35.5
eGFR (ml/min/1.73 m^2)	73.6 ± 19.2	80.7 ± 25.6
GFR < 60 ml/min/1.73 m^2 (%)	19.2	19.8
ACR	1.3 (0.6–4.0)	2.0 (0.7–8.3)
Micro-/macro-albuminuria (%)	28.4	38.2
Adiponectin (µg/ml)	6.0 ± 3.6	6.2 ± 3.8
Resistin (ng/ml)	10.3 ± 8.0	8.3 ± 6.0

Continuous variables were reported as mean ± SD, or median (interquartile range) for skewed variables, whereas categorical variables were reported as percentages.

GMS: Gargano Mortality Study; FMS: Foggia Mortality Study; HbA1c: glycated hemoglobin; TX: treatment; eGFR: estimated glomerular filtration rate; ACR: albumin creatinine ratio.

In GMS, during follow-up (10.5 ± 3.7 years, 9,397 py), 290 deaths occurred, corresponding to an annual IR of 3.1%. In FMS, during follow-up (7.1 ± 2.5 years, 3,685 py), 140 deaths occurred, corresponding t an annual incidence rate of 3.8%.

In both studies, each SD increase of log transformed adiponectin [HRs; 95% CI = 1.38 (1.23–1.55); $p = 4.30 \times 10^{-8}$ and 1.59 (1.32–1.91); $p = 1.22 \times 10^{-6}$, in GMS and FMS, respectively] and log transforme resistin [(HRs; 95% CI = 1.42 (1.27–1.59); $p = 6.81 \times 10^{-10}$ and 1.37 (1.17–1.59); $p = 8.28 \times 10^{-5}$, in GMS and FMS, respectively], were significantly associated with all-cause mortality. No difference in effect sizes across the two studies were observed (p values for within-study heterogeneity equal to 0.22 and 0.73 for adiponectin and resistin, respectively). GMS and FMS were then pooled, so to increase statistical power i investigating the nature of the joint effect of the two adipokines on mortality rate. In the whole sample, adiponectin and resistin were associated with all-cause mortality, independent of each other (Table 2, model 1) Such associations did not change much after including different covariates, known to influence *per se* mortality rate (Table 2, models 2–6).

Table 2 Association between both serum adiponectin and resistin and all-cause mortality in the pooled sample (N = 1,414).

	Adiponectin		Resistin	
	HR (95% CI)	*p*	HR (95% CI)	*p*
Model 1	1.40 (1.27–1.55)	1.03×10^{-11}	1.38 (1.26–1.51)	4.59×10^{-12}
Model 2	1.14 (1.03–1.27)	0.012	1.31 (1.20–1.43)	3.59×10^{-9}
Model 3	1.17 (1.05–1.31)	0.004	1.30 (1.18–1.42)	5.29×10^{-8}

Model 4	1.17 (1.05–1.31)	0.005	1.27 (1.16–1.40)	5.25×10^{-7}
Model 5	1.15 (1.03–1.29)	0.014	1.20 (1.09–1.33)	3.94×10^{-4}
Model 6	1.18 (1.05–1.32)	0.004	1.30 (1.17–1.43)	4.34×10^{-7}

HR (95% CI) are given for the increase of 1SD of log transformed values of serum adiponectin and resistin.

Model 1: including both adipokines and adjusted for "study sample" (i.e. GMS and FMS).

Model 2: adjusted as in Model 1 plus sex, age at recruitment and smoking habits (i.e. general confounders).

Model 3: adjusted as in Model 2 plus BMI and waist circumference (i.e. adiposity-related confounders).

Model 4: adjusted as in Model 3 plus, HbA1c, diabetes duration and age at diabetes diagnosis (i.e. diabetes-related confounders). In this case, age at recruitment was excluded from the analysis because of its collinearity with diabetes duration and was replaced by age at diagnosis.

Model 5: adjusted as in Model 3 plus, eGFR and ACR (i.e. kidney function-related confounders).

Model 6: adjusted as in Model 3 plus glucose-lowering, anti-hypertensive, anti-dyslipidemic treatments (i.e. ongoing treatment-related confounders).

Of note, no evidence of adiponectin-by-resistin interaction was observed ($p = 0.40$), thus speaking against a synergistic and in favor of an additive effect of the two adipokines on all-cause mortality.

To get deeper insights on such joint effect, the pooled sample was stratified according to relatively high and low adiponectin and resistin levels (i.e. above or below the median value, being 5.14 µg/ml an 7.71 ng/ml, respectively). Then, four groups were obtained: low/low, high/low; low/high and high/high adiponectin/resistin levels from now named as group 1, 2, 3 and 4, respectively. IR of all-cause mortality was 1.9% (69 events/3,608 py), 3.2% (98 events/3,045 py), 3.2% (100 events/3,078 py), and 4.9% (163 events/3,337 py), in groups 1–4, respectively. Survival probabilities of the four groups are shown in Fig. 1. As compared t group 1, the HRs for all-cause mortality were 1.81 (1.33–2.47); $p = 1.73 \times 10^{-4}$, 1.97 (1.44–2.69); $p = 2.33 \times 10^{-5}$ and 3.02 (2.26–4.03); $p = 9.21 \times 10^{-14}$, in group 2, 3 and 4, respectively. HR in group 4 was significantl higher than that in group 2 and 3 ($p = 1.46 \times 10^{-4}$ and $p = 0.001$, respectively). Clearly, no difference were observed between group 2 and 3 ($p = 0.45$). In all, these analyses, carried out by comparing differen subgroups, confirm that adiponectin and resistin exert an additive effect in shaping the risk of all-cause mortality.

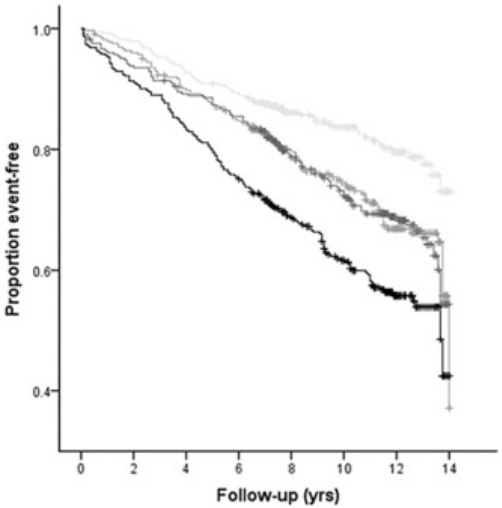

Fig. 1 Kaplan-Meier survival curves for all-cause mortality in the combined sample (GMS + FMS) according to the four groups with relatively high and low adiponectin and resistin levels (i.e. above or below the median value, being 5.14 µg/ml and 7.71 ng/ml, respectively). Group 1 - light grey- (low adiponectin/low resistin; N = 383; 69 events), group 2 - silver- (high adiponectin/low resistin; N = 325; 98 events), group 3 -grey- (low adiponectin/high resistin; N = 325; 100 events) and group 4 - black- (high adiponectin/high resistin; N = 381; 163 events).

We then tested whether the highly increased mortality risk in group 4 *vs.* group 1 was modulated by synergistic interaction with various demographic and clinical features. To this purpose, mortality risk wa compared across subgroups in a multivariable model considering general confounders, such as sex, age at recruitment and smoking habits (Fig. 2). The increased risk in group 4 was significantly more pronounced i individuals with relatively low waist circumference values (p for HR heterogeneity = 0.03) (Fig. 2). Such modifying effect was toward the same direction in both GMS and FMS, though reaching formal statistica significance only in the former sample (i.e. $p = 0.04$ and 0.17, respectively). Of note, in the interactive model, waist circumference *per se* revealed a neutral effect on mortality rate (HR, 95% CI = 1.00, 0.99–1.01). N evidence of synergism was observed with any of the other demographic and clinical features (Fig. 2).

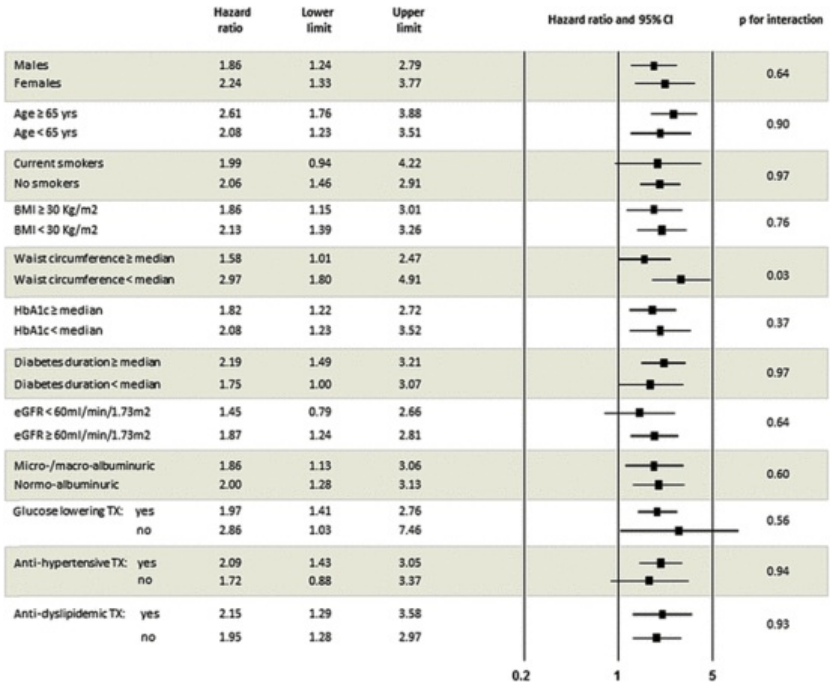

Fig. 2 Hazard ratios (95% CI) of all-cause mortality in group 4 (high adiponectin/high resistin levels) *vs.* (low adiponectin/low resistin levels) ~~per one SD increment of natural logarithm transformed adiponectin and resistin levels, in the combined sample (GMS + FMS)~~ by subgroups of demographical and clinical features. Age at recruitment, sex and smoking habits adjusted HRs were estimated by Cox regression. The p value for interaction (i.e. multiplicative effect) is shown for each subgroup. Median for waist circumference was 101.0 cm for males and 103.0 cm for females; median for HbA1c was 8.5%; median for diabetes duration was 10 yrs. Increased albuminuria was diagnosed if the urinary albumin/creatinine ratio was ≥2.5 mg/mmol in me and ≥3.5 mg/mmol in women.

4 Discussion

The main finding of our study, carried out in 1,414 patients with T2D, is that adiponectin and resistin exert additive effects on mortality rate, which are independent of each other. The magnitude of such join effect, which is also independent by several possible confounders, is of utmost epidemiological and clinical significance, with patients with the highest serum concentrations of both adipokines showing a tripled risk c death as compared to that of patients with the lowest levels. Our data are at variance with those from Spoto et al. [21] reporting, in 231 hemodialysis patients, that the joint effect of adiponectin and resistin on all-caus mortality was synergic with the deleterious role of high resistin levels being observable only among individuals with low serum adiponectin [21]. The reasons for such discrepancy are not known. Differences in th clinical set (i.e. diabetic patients in our case *vs.* hemodialysis patients in Spoto's study), in study design (i.e. considering all baseline treatments only in our study) and in sample size magnitude may well explain th different results. It is also of note considering that while the role of the two adipokines, as singly considered, on mortality rate is established in several clinical sets, including T2D [3,4,6,12–20], this is definitively no the case in hemodialysis patients, where such role is still uncertain [21,32–38].

The biology underlying the additive effect we observed is unknown and cannot be certainly unraveled by an observational study like the present one, which allows only offering some speculations. While the rol

of resistin on mortality is compatible with its pro-inflammatory, pro-atherogenic role [8,9,39], the paradoxical association between high adiponectin and mortality is counterintuitive. Until now, the only assumptio offered was that adiponectin increases in high risk individuals, as an unsuccessful protective mechanism either due to adiponectin resistance [40–42] or due to increased N-terminal pro brain natriuretic peptide (NT proBNP) [43]. However, our recent data, obtained with a Mendelian randomization approach, point to a cause-effect relationship between high adiponectin and increased rate of cardiovascular death [44]. Recent dat reporting pro-atherogenic adiponectin effects, including angiogenesis [45,46] and inflammatory cytokines secretion [45,47,48] offer mechanistic support to our finding [44]. In all, while several intervention tools ar available in order to reduce serum resistin concentration [49–53], it is unfortunate this is not the case for serum adiponectin levels, thus impeding to test in a clinical trial whether the reduction of both adipokines level is associated with reduced mortality rate.

Our present data are compatible with the idea that adiponectin, and resistin mainly act through independent and distinct pathways [54,55], though recent animal studies in rats [56,57] have questioned thi scenario, by providing some evidence of interaction between signaling pathways of the two adipokines. Waist circumference modulates the joint additive effect of adiponectin and resistin on mortality rate, which, i fact, was more pronounced in individuals with relatively reduced abdominal adiposity. This is at variance with what previously reported in hemodialysis patients in whom, however, a protective, rather than deleterious, effect of high adiponectin levels was observed [32]. The biology underlying the synergistic effect we observed is unknown and is difficult to explain. Also in this case, the observational nature of our stud does not provide any mechanistic information; as said before, while there are several tools able to reduce serum resistin levels, this is not the case for circulating serum adiponectin, thus making impossible to tes whether or not the combined serum changes of these two adipokines affect the risk of mortality, independently of abdominal adiposity. We can here only speculate that in patients with high waist circumference othe risk factors intrinsically linked with excess abdominal adiposity, either of environmental [58–61] or of genetic origin [62–64], have masked the additive effect of the two adipokines. Although very similar data wer obtained in both GMS and FMS, we do acknowledge that the statistical level reached by the interaction analysis imposes caution in data interpretation and indicate the need of further studies aimed at bette addressing the modifying effect of abdominal adiposity on the combined deleterious role of adipokines on mortality rate.

Strength of our study is the use of well-established prospectively analyzed cohorts [12,22–25] with a completeness of information, including standardized clinical evaluations. In addition, the two studies wer carried out in the same geographical region, with identical inclusion/exclusion criteria, with all samples handled identically and with serum adipokines measurement being centralized.

A major limitation of our study is intrinsic to its very nature; in fact, as an observational study it does not provide mechanistic information, leaving, therefore, unanswered any question about the biolog underlying our finding.

A further limitation is the clear baseline clinical heterogeneity we observed across the two study samples. Despite this, no difference was observed across the two cohorts either in the rate of, or in th adipokines effects on, all-cause mortality, our unique study end point. Nonetheless, when running pooled analyses, we were conservative enough to adjust for “study sample”, thus taking into account all baselin differences across studies.

A second limitation is that no data on cause-specific mortality were available, thus making impossible to extend our observation to specific causes of death.

Finally, it is not known whether or not our present finding is generalizable to populations of different ethnicity with different environmental and/or genetic background and/or to non-diabetic individuals.

In conclusion, to the best of our knowledge, this is the first study reporting an additive independent effect of increased adiponectin and resistin on all-cause mortality in a large sample of patients with T2D. Suc additive effect is modulated by abdominal adiposity.

Further studies are definitively needed to: i) confirm the modifier effect of abdominal adiposity on this association; ii) verify whether adding these two adipokines to previously validated prediction tools c clinical relevance [23] turns to be useful in improving prediction ability of mortality rate in diabetic patients; iii) explore if intervention studies aimed at reducing both adipokines decrease the risk of mortality in suc patients.

Conflict of interest

The authors declared that they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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Author contributions

L.O.M., V.T. and C.M. participated in study concept and design, acquisition of data, interpretation of results and drafted the manuscript. L.O.M. A.F. and M. Copetti participated in statistical analysis. O.L., L.S C.D.B., and M. Cignarelli provided essential data for analyses. All authors read and approved the final version of the manuscript.

V.T. and C.M. are the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data.

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Highlights

- Adiponectin and resistin have additive independent effects on mortality rate in T2D.
- Abdominal fat is the only clinical feature that modulates such combined effect.
- This joint effect is more evident in patients with relatively low waist circumference.

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