Efficacy of Antioxidant Treatment in Reducing Resistin Serum Levels: A Randomized Study

Simona Bo1*, Giovannino Ciccone2, Marilena Durazzo1, Roberto Gambino1, Paola Massarenti3, Ileana Baldi2, Antonela Lezo1, Elisa Tiozzo3, Daniela Pauletto3, Maurizio Cassader1, Gianfranco Pagano1

1 Department of Internal Medicine, University of Turin, Turin, Italy, 2 Unit of Cancer Epidemiology, University of Turin and Centro di Riferimento per l’Epidemiologia e la Prevenzione Oncologica in Piemonte, Turin, Italy, 3 Laboratory of Clinical Nutrition, San Giovanni Battista Hospital, Turin, Italy

Trial Registration: NCT00387114

Funding: This study was supported by a grant from Regione Piemonte, 2004. The Unit of Cancer Epidemiology received a grant from the San Paolo Company, to support randomized controlled trials. The sponsors had no role in study design; collection, analysis, and interpretation of data; writing of the paper; or decision to submit it for publication.

Competing Interests: The authors have declared that no competing interests exist.


Received: October 13, 2006
Accepted: February 19, 2007
Published: May 4, 2007

Copyright: © 2007 Bo et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Abbreviations: CI, confidence interval; CRP, C-reactive protein; CV, coefficient of variation; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; NO, nitric oxide; NT, nitrotyrosine

* To whom correspondence should be addressed. E-mail: sbob@molinette.piemonte.it

ABSTRACT

Objectives: Few in vitro studies have examined the participation of resistin, a recently discovered adipokine, in oxidative processes. We investigated whether in vivo treatment with the antioxidant vitamin C might affect resistin serum levels.

Design: Randomized prospective open trial.

Setting: San Giovanni Battista Hospital, Turin, Italy.

Participants: Eighty healthy individuals.

Intervention: Administration of 2 g of ascorbic acid orally for 2 wk (n = 40; experimental group) or no supplementation (n = 40; control group).

Outcome measures: The primary end point was the between-group difference in the before–after change in resistin serum level after vitamin C supplementation. Secondary endpoints were the within- and between-group changes in glucose, insulin, lipid parameters, C-reactive protein fasting values, and markers of oxidative stress.

Results: In the experimental group, vitamin C supplementation was significantly associated with both resistin concentration reduction (from 4.3 ± 1.5 to 2.9 ± 0.8 ng/ml; 95% confidence interval [CI] −1.87, −1.03) and ascorbic acid level increase (from 9.4 ± 2.9 to 19.0 ± 5.2 mg/l; 95% CI 7.9, 11.2). In the control group, resistin levels did not change significantly (from 4.2 ± 1.0 to 4.3 ± 0.9 ng/ml; 95% CI −0.07, 0.37). The between-group differences were highly significant (p < 0.001). Vitamin C supplementation was also associated with a statistically significant reduction in nitrotyrosine level and incremental increase in reduced glutathione. In a linear regression model, within-individual changes in vitamin C concentrations were inversely correlated with changes in resistin levels in both groups (each unit increase of vitamin C corresponded to a decrease of about 0.10 units of resistin levels (95% CI 0.13, 0.08; p < 0.001).

Conclusion: This is to our knowledge the first randomized trial in humans that has demonstrated that short-term vitamin C supplementation could significantly reduce resistin levels, independent of changes in inflammatory or metabolic variables. Future investigations of resistin participation in oxidative processes are warranted.
Resistin, a recently described adipokine belonging to the cysteine-rich secretory protein family, was originally described as an adipocyte-derived polypeptide that links obesity and insulin resistance in mice [1]. However, in humans, resistin is expressed at very low concentrations in adipose cells [2], but at high levels in mononuclear leukocytes, macrophages, spleen cells, and bone marrow cells [3]. Striking differences in the genomic organization and cellular source of resistin in rodents versus humans make the biological effects found in the mouse not readily transferable to humans [4–6]. Accordingly, an increasing number of reports have raised doubts regarding the possibility of an important relationship between human resistin and various metabolic disturbances characteristic of obesity and type 2 diabetes [7–13].

Resistin was originally found to be related to proteins induced during lung inflammation [14], and the likelihood that it may be involved in the inflammation process [4,12,15–22] is suggested by the high expression levels of resistin in leukocytes, the associations between this protein and inflammatory markers, and the resistin’s ability to stimulate in vivo inflammatory cytokines.

Data supporting resistin participation in oxidative processes have been sporadically published. Significant interaction between a single nucleotide polymorphism in the promoter of the human resistin gene and a marker of oxidative stress (NAD(P)H:quinone oxidoreductase 1) has been found [23]. Retinoic acid, the acid form of vitamin A, inhibited the expression of resistin in mice and reduced the higher resistin levels of ten men affected by psoriasis [24,25]. Resistin serum levels were inversely associated with nitrotyrosine (NT), a product of free radical activity [22]; resistin inhibited endothelial nitric oxide synthase activation in vitro, thus reducing nitric oxide (NO) bio-availability [26].

The aim of this explicative trial was to evaluate whether an in vivo short-term treatment with an antioxidant vitamin (vitamin C) might substantially affect resistin serum levels. For this purpose, serum resistin values were evaluated in a group of healthy participants, randomized to receive orally 2 g of ascorbic acid daily for 2 wk. Values of fasting glucose, insulin, total and high-density lipoprotein (HDL) cholesterol, triglycerides, and C-reactive protein (CRP) and markers of oxidative stress were measured.

METHODS
Participants
After obtaining approval from the San Giovanni Battista Hospital Ethical Committee and informed written consent from participants, a randomized prospective open trial was carried out. Healthy European-descent volunteers aged 20–50 y were recruited from the staff of the San Giovanni Battista Hospital in Turin. Exclusion criteria were as follows: current pregnancy; hyperglycemia (fasting glucose ≥ 6.1 mmol/l); hypertension (blood pressure ≥ 140/90 mm Hg); impaired renal function (serum creatinin ≥ 106 μmol/l); known cardiovascular disease, liver disease, or any other systemic conditions; use of any drug (estrogen included); and being on a particular diet and/or vitamin or other nutrient supple-
ments. All procedures conformed to the principles of the Declaration of Helsinki.

Outcomes
The primary end point was the between-group difference in the before–after change in resistin serum levels after 2 wk of vitamin C treatment in the experimental group. Secondary end points were the within- and between-group comparisons of changes in the other inflammatory and oxidative variables measured.

Sample Size
On the basis of our previous data [22], a sample of at least 34 individuals per group was required to detect a standardized difference of 0.5 standard deviations in resistin levels between groups, with a statistical power of 80% and a two-tailed 0.05 α-value. Taking into account the limitations of these assumptions and the possibility of missing some individuals, the total sample size was increased to 40 individuals per group.

Recruitment
In June–July 2005, a total of 80 eligible participants were enrolled and submitted in the morning, at fasting, to measurements of weight, waist circumference, and blood pressure, and to determinations of serum glucose, total cholesterol, HDL cholesterol, triglyceride, insulin, high-sensitivity CRP (hs-CRP), vitamin A, vitamin C, vitamin E, oxidized and reduced glutathione, NT, and resistin levels. None of the participants showed impaired values of blood pressure, glucose, or cholesterol. Data were collected on smoking habits and physical activity via an interview, and on mean daily nutrient intake via a 3-d food record.

Interventions in the two arms were either daily 2 g of ascorbic acid supplementation or no supplementation for 14 d. Tablets of 1 g of ascorbic acid were consumed twice a day, one at fasting upon awakening and the second 12 h later, every day for 14 consecutive days. All the participants were advised to continue their habitual diet and perform their usual physical activity.

The second blood sample, likewise after 8–12 h of fasting, was collected from the two groups in September–November 2005. The date of the second assessment was fixed in advanced for all participants, well before treatment began for the experimental group, so that any influence of the condition of the participant or his eating patterns on the date of assessment can be ruled out.

In the experimental group a blood sample was collected the morning after the last vitamin C tablet had been taken. During the time lag between the first (June–July 2005) and the second assessment (September–November 2005) no change in health status occurred; participants maintained their weight, waist circumference, and blood pressure values. None of the participants started any treatment (including anti-inflammatory drugs) or were affected by any pathological conditions, including acute or chronic inflammatory conditions or infections.

Randomization: Sequence Generation
Participants were stratified according to age, sex, smoking habits, body mass index, and fasting levels of glucose, hs-CRP, vitamin C, and resistin. After collection of all baseline data for all participants, the randomization procedure was automatically performed by a statistician, using a SAS program developed to minimize the differences between the two groups for all the stratifying variables. The final distribution was 40 participants on vitamin C supplementation (experimental group) and 40 not supplemented (control group).

Randomization: Allocation Concealment
When the results of baseline tests were available for all 80 participants, random allocation with a minimization algorithm was centrally performed in a single step. The researchers received then two lists of nominative data (40 for the experimental group and 40 for the control group). In this way the possibility for researchers to predict or influence the allocation of participants was completely prevented.

Measurements
The 3-d food record consisted of a detailed written food diary kept prospectively: the participants were instructed to record everything they ate or drank during two consecutive week days and one weekend day.

Weight and waist and hip circumference were measured using standard protocols. Systolic and diastolic blood pressures were measured twice with a standard mercury sphygmomanometer in a sitting position, after at least 10 min of rest. Values reported are the mean of the two determinations.

Physical activity during leisure time was defined as light (inactive, <4 h/wk), moderate (4 h/wk), or heavy (≥4 h/wk) [27].

Serum glucose was measured by the glucose oxidase method, and triglycerides and HDL cholesterol by enzymatic colorimetric assay (Hitachi 911 Analyzer, Sentinel, Milan, Italy). Serum insulin values were determined by immunoradiometric assay (Radim, Pomezia, Italy; intra-assay coefficient of variation (CV) 1.9%, inter-assay CV 6.2%). Serum CRP levels were measured by a high-sensitivity latex agglutination method (hs-CRP) on the Hitachi 911 Analyzer (intra-assay and inter-assay CVs respectively 0.95% and 1.3%). Serum NT values were determined by an ELISA kit (HyCult Biotechnology Pantec, Turin, Italy; intra-assay precision 3.3%; intra-assay CV 5%). Serum resistin values were analyzed by ELISA (BioVendor, Brno, Czech Republic); the intra-assay and inter-assay CVs were, respectively, 2.8% and 5.5%.

Plasma vitamin C levels were detected by HPLC (Solvent Delivery System 9012, with a UV-Visible Detector 9050, Varian, Walnut Creek, California, United States); intra-assay and inter-assay CVs respectively 4.8% and 5.9%). Plasma vitamin A and vitamin E were simultaneously evaluated by HPLC (Chromsystems Instruments, Munich, Germany; intra-assay and inter-assay CVs respectively 3.3% and 5.6%). Total and reduced glutathione in red blood cells were analyzed by HPLC after a derivatization of hemolyzed samples with ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulphonate, using a fluorescence detector (Varian 9070) with excitation at 385 nm and emission at 515 nm. Oxidized glutathione was calculated as the difference between total and reduced glutathione (intra-assay and inter-assay CVs respectively 4.1% and 5.3%).

All blood samples underwent laboratory testing in blind.
RESULTS

Participant Flow

Out of the 40 participants in the control group, two were lost during follow-up (they moved away). No participant discontinued treatment or was lost during follow-up in the experimental group. Data from 78 participants were thus analyzed. The flow diagram of the trial is reported in Figure 1.

Baseline Data

Basal clinical and laboratory characteristics of all the enrolled participants by group are shown in Table 1. No meaningful difference was evident between the two groups. Habitual nutrient intake patterns were very similar between the two groups; in particular, estimated vitamin C intake from food was 142.5 ± 77.2 and 144.8 ± 70.4 mg/dl, respectively, in the experimental and control groups. Similarly, intake of total calories; total fat; polyunsaturated, saturated, and monounsaturated fat (as energy percent); fiber; and vitamin A and E were not significantly different between the two groups (data not shown).

Outcomes and Estimation

After vitamin C supplementation, ascorbic acid plasma levels significantly increased in the experimental group, while a slight reduction was observed in the control group (Table 2). Resistin serum concentrations showed a significant reduction in the experimental group (from 4.3 to 2.9 ng/ml) and a small, not significant, increase in the control group (from 4.2 to 4.3 ng/ml). The differences between groups in the blood levels of vitamin C and resistin after supplementation were highly significant ($p < 0.001$) (Table 2). Reduced glutathione concentrations increased in both groups, but the increment was more than 2-fold higher in the experimental group. Levels of NT significantly decreased in the experimental group, and slightly increased in the controls, giving a between-group difference of borderline statistical significance. Values of hs-CRP slightly increased in the controls, and no significant change was evident for fasting glucose, insulin, lipid variables, or other antioxidant vitamins within or between groups.

Figure 2 shows the standardized between-group differences (and 95% CI) for the evaluated blood variables. Apart from the large differences recorded for vitamin C and resistin concentrations, only NT and reduced glutathione variations were affected by the antioxidant treatment. According to the results of the robust linear regression, changes in resistin serum levels were inversely related to changes in vitamin C plasma levels both in the experimental and control groups. On the whole sample, a one-unit increase in before–after change of vitamin C corresponded to a statistically significant decrease ($\beta = -0.10; 95\% \, CI \, -0.13, -0.08; \, p < 0.001$) in the mean change of resistin. This overall relationship still holds after adjusting by group ($\beta = -0.08; 95\% \, CI \, -0.13, -0.03; \, p = 0.003$).

Figure 3 reports the observed and predicted values, with 95% confidence bands, estimated for both experimental and control groups.

Adverse Events

Two participants in the experimental group complained of gastric discomfort after vitamin C supplementation.
The results of this trial clearly indicated that in healthy individuals resistin serum levels were significantly reduced by a short period of supplementation with vitamin C. This treatment was also associated with other changes in oxidation-related variables (reduction of NT levels and incremental increase of reduced glutathione).

To date, there has been considerable controversy surrounding the physiological relevance of resistin: the associations with insulin resistance and obesity, reported in mice, have not been conclusively demonstrated in humans [7–13]. Growing evidence has suggested that this protein, highly expressed in monocytes and macrophages in humans, might have an involvement in the inflammation process, either being induced by increased cytokine levels, or directly stimulating the production of pro-inflammatory cytokines, thus leading to inflammation amplification [4,12–22]. Recent studies found that resistin activated human endothelial cells in vitro, inducing the expression of endothelin 1, adhesion molecules, and chemokines [16,30], and stimulated smooth muscle cell proliferation [31]. Endothelial dysfunction and/or pro-inflammatory effects could represent, thus, the link between higher resistin serum levels and increased prevalence of cardiovascular diseases in humans. This latter association was demonstrated by some studies [20,32,33] and supported by the finding of elevated resistin secretion from macrophages infiltrating atherosclerotic arterial walls [34].

It was recently demonstrated that resistin impairs endothelium-dependent vasorelaxation in porcine coronary arteries, by reducing NO bio-availability [35]. Possible participation of resistin in oxidative processes has been sporadically suggested [22–26], and an impact of oxidative stress on the regulation of murine adipokine gene expression was proposed [36]. The possibility that antioxidants might act on resistin expression and levels has been suggested by a few studies on animals: retinoic acid inhibits the expression of resistin in mice [24], and the antioxidant seleno-methionine, which increases the activity of glutathione peroxidase in endothelial cells, completely blocks vasomotor dysfunction induced by resistin in porcine arteries [35]. In humans, a small study testing the effect of a chronic treatment with retinoid therapy on patients with psoriasis showed normalization of the increased resistin serum levels, together with a reduction in insulin sensitivity, without any changes in circulating adiponectin or tumor necrosis factor concentrations [25].

Our data clearly show that the antioxidant supplementation significantly reduced resistin serum levels, but changed neither metabolic parameters nor hs-CRP levels (Table 2), the latter being, however, already very low at baseline. In the control group, unexpectedly, we observed a slight, statistically significant reduction in vitamin C plasma levels and a small

<table>
<thead>
<tr>
<th>Variables</th>
<th>Variable Specifications</th>
<th>Experimental Group (n = 40)</th>
<th>Control Group (n = 40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>34.4 ± 8.1</td>
<td>34.0 ± 8.0</td>
<td></td>
</tr>
<tr>
<td>Age groups (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20–30 y</td>
<td>45.0</td>
<td>47.5</td>
<td></td>
</tr>
<tr>
<td>31–40 y</td>
<td>25.0</td>
<td>22.5</td>
<td></td>
</tr>
<tr>
<td>41–50 y</td>
<td>30.0</td>
<td>30.0</td>
<td></td>
</tr>
<tr>
<td>Males (%)</td>
<td>30.0</td>
<td>32.5</td>
<td></td>
</tr>
<tr>
<td>Non-smoking (%)</td>
<td>87.5</td>
<td>87.5</td>
<td></td>
</tr>
<tr>
<td>Education level (%)</td>
<td>University</td>
<td>62.5</td>
<td>55.0</td>
</tr>
<tr>
<td>Secondary school</td>
<td>30.0</td>
<td>37.5</td>
<td></td>
</tr>
<tr>
<td>Physical activity (%)</td>
<td>Light</td>
<td>62.5</td>
<td>65.0</td>
</tr>
<tr>
<td>Moderate</td>
<td>32.5</td>
<td>27.5</td>
<td></td>
</tr>
<tr>
<td>Heavy</td>
<td>5.0</td>
<td>7.5</td>
<td></td>
</tr>
<tr>
<td>Alcohol (g/d)</td>
<td>2.5 ± 5.7</td>
<td>2.0 ± 5.5</td>
<td></td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.68 ± 0.1</td>
<td>1.69 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)a</td>
<td>61.5 ± 12.7</td>
<td>63.0 ± 12.2</td>
<td></td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>73.9 ± 12.0</td>
<td>75.6 ± 10.9</td>
<td></td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.79 ± 0.09</td>
<td>0.80 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>21.7 ± 3.0</td>
<td>22.1 ± 2.4</td>
<td></td>
</tr>
<tr>
<td>Systolic pressure (mm Hg)</td>
<td>118.0 ± 11.6</td>
<td>119.4 ± 14.0</td>
<td></td>
</tr>
<tr>
<td>Diastolic pressure (mm Hg)</td>
<td>74.9 ± 7.4</td>
<td>74.5 ± 7.7</td>
<td></td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>4.7 ± 0.5</td>
<td>4.6 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.9 ± 0.8</td>
<td>4.9 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.4 ± 0.3</td>
<td>1.5 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mmol/l)b</td>
<td>0.8 (6.0)</td>
<td>0.8 (0.3)</td>
<td></td>
</tr>
<tr>
<td>Fasting insulin (pmol/l)b</td>
<td>80.4 (56.4)</td>
<td>81.9 (58.8)</td>
<td></td>
</tr>
<tr>
<td>CRP (mg/l)b</td>
<td>0.6 (1.0)</td>
<td>0.6 (0.8)</td>
<td></td>
</tr>
<tr>
<td>Resistin (ng/ml)</td>
<td>4.3 ± 1.5</td>
<td>4.2 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>Vitamin C (mg/l)</td>
<td>9.4 ± 2.9</td>
<td>9.3 ± 2.3</td>
<td></td>
</tr>
<tr>
<td>Vitamin A (mg/l)</td>
<td>0.45 ± 0.13</td>
<td>0.47 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>Vitamin E (mg/l)</td>
<td>12.0 ± 3.2</td>
<td>12.9 ± 3.7</td>
<td></td>
</tr>
<tr>
<td>Reduced glutathione (µmol/l)</td>
<td>1,428.6 ± 271.7</td>
<td>1,492.9 ± 352.8</td>
<td></td>
</tr>
<tr>
<td>Oxidized glutathione (µmol/l)</td>
<td>333.0 ± 124.5</td>
<td>358.2 ± 123.8</td>
<td></td>
</tr>
<tr>
<td>NT (nmol/ml)</td>
<td>16.7 ± 5.3</td>
<td>15.0 ± 4.5</td>
<td></td>
</tr>
</tbody>
</table>

aRanges respectively 41–98 kg and 43–93 kg in the experimental and control groups.
bMedian ± interquartile range for non-normally distributed values.

doi:10.1371/journal.pctr.0020017.t001

DISCUSSION

Interpretation

The results of this trial clearly indicated that in healthy individuals resistin serum levels were significantly reduced by a short period of supplementation with vitamin C. This treatment was also associated with other changes in oxidation-related variables (reduction of NT levels and incremental increase of reduced glutathione).

To date, there has been considerable controversy surrounding the physiological relevance of resistin: the associations with insulin resistance and obesity, reported in mice, have not been conclusively demonstrated in humans [7–13]. Growing evidence has suggested that this protein, highly expressed in monocytes and macrophages in humans, might have an involvement in the inflammation process, either being induced by increased cytokine levels, or directly stimulating the production of pro-inflammatory cytokines, thus leading to inflammation amplification [4,12–22]. Recent studies found that resistin activated human endothelial cells in vitro, inducing the expression of endothelin 1, adhesion molecules, and chemokines [16,30], and stimulated smooth muscle cell proliferation [31]. Endothelial dysfunction and/or pro-inflammatory effects could represent, thus, the link between higher resistin serum levels and increased prevalence of cardiovascular diseases in humans. This latter association was demonstrated by some studies [20,32,33] and supported by the finding of elevated resistin secretion from macrophages infiltrating atherosclerotic arterial walls [34].

It was recently demonstrated that resistin impairs endothelium-dependent vasorelaxation in porcine coronary arteries, by reducing NO bio-availability [35]. Possible participation of resistin in oxidative processes has been sporadically suggested [22–26], and an impact of oxidative stress on the regulation of murine adipokine gene expression was proposed [36]. The possibility that antioxidants might act on resistin expression and levels has been suggested by a few studies on animals: retinoic acid inhibits the expression of resistin in mice [24], and the antioxidant seleno-methionine, which increases the activity of glutathione peroxidase in endothelial cells, completely blocks vasomotor dysfunction induced by resistin in porcine arteries [35]. In humans, a small study testing the effect of a chronic treatment with retinoid therapy on patients with psoriasis showed normalization of the increased resistin serum levels, together with a reduction in insulin sensitivity, without any changes in circulating adiponectin or tumor necrosis factor concentrations [25].

Our data clearly show that the antioxidant supplementation significantly reduced resistin serum levels, but changed neither metabolic parameters nor hs-CRP levels (Table 2), the latter being, however, already very low at baseline. In the control group, unexpectedly, we observed a slight, statistically significant reduction in vitamin C plasma levels and a small
increase in serum resistin values (Figure 3). This relationship was independent from changes in other metabolic/inflammatory parameters, in line with the absence of correlations between tumor necrosis factor and resistin changes observed in males with psoriasis who received retinoid treatment [25]. This suggested a specific direct effect of antioxidant substances on resistin expression from monocytes/macrophages. Accordingly, the reduction in resistin levels was maintained 3 mo after retinoid treatment in males with psoriasis, unlike the transient effect found by the authors on insulin sensitivity [25].

It has been suggested that resistin inhibits endothelial nitric oxide synthase activation in vitro and increases superoxide radical production in porcine coronary arteries [35] and human endothelial cells [26], thus reducing NO bioavailability. Imbalance in the production and regulation of oxygen radicals and the subsequent oxidative inactivation of NO leads to oxidative stress and might contribute to vascular disease [36].

The reaction of NO with superoxide anion radicals (O$_2^-$•) yields peroxynitrite (ONOO$^-$), which can oxidize many biomolecules. NT, generated from the oxidation of tyrosine, has been considered as a measure of ONOO$^-$ oxidative injury, and elevated plasma levels have been reported in conditions associated with oxidative stress, such as diabetes [37–39]. In the experimental group, NT levels were significantly reduced after the supplementation, while a very slight increase was evident in the control group. These changes might be due to the direct protective antioxidant effect of vitamin C. Ascorbic acid could stimulate reduced glutathione synthesis, and, thus, might be responsible for the statistically significant incremental increase in its levels found in the experimental group [40].

Our study is to our knowledge the first human randomized trial carried out on a very homogeneous sample of healthy volunteers that has demonstrated that short-term supplementation with an antioxidant vitamin might significantly reduce resistin serum levels, independent of changes in inflammatory and metabolic variables. This finding, together with in vitro results [26,35], indicates that oxidative stress may be one of the major mechanisms by which resistin acts and is

<table>
<thead>
<tr>
<th>Blood Variable</th>
<th>Experimental Group (n = 40)</th>
<th>Control Group (n = 38)</th>
<th>p-Value$^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Difference</td>
</tr>
<tr>
<td>Vitamin C (mg/l)</td>
<td>9.4 ± 2.9</td>
<td>19.0 ± 5.2</td>
<td>9.55</td>
</tr>
<tr>
<td>Resistin (ng/ml)</td>
<td>4.3 ± 1.5</td>
<td>2.9 ± 0.8</td>
<td>−1.45</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>4.7 ± 0.5</td>
<td>4.7 ± 0.5</td>
<td>0.00</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.9 ± 0.8</td>
<td>4.9 ± 0.9</td>
<td>−0.03</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.4 ± 0.3</td>
<td>1.4 ± 0.3</td>
<td>0.006</td>
</tr>
<tr>
<td>Log triglycerides (mmol/l)$^b$</td>
<td>−0.1 ± 0.6</td>
<td>−0.1 ± 0.4</td>
<td>0.01</td>
</tr>
<tr>
<td>Log fasting insulin (mmol/l)$^b$</td>
<td>4.5 ± 0.6</td>
<td>4.4 ± 0.3</td>
<td>−0.07</td>
</tr>
<tr>
<td>Log CRP (mg/l)$^b$</td>
<td>−0.6 ± 1.0</td>
<td>−0.6 ± 1.1</td>
<td>0.04</td>
</tr>
<tr>
<td>Vitamin A (mg/l)</td>
<td>0.45 ± 0.13</td>
<td>0.48 ± 0.15</td>
<td>0.03</td>
</tr>
<tr>
<td>Vitamin E (mg/l)</td>
<td>12.0 ± 3.2</td>
<td>12.7 ± 3.5</td>
<td>0.68</td>
</tr>
<tr>
<td>Reduced glutathione (μmol/l)</td>
<td>1,428.6 ± 271.7</td>
<td>1,789.0 ± 415.1</td>
<td>360.4</td>
</tr>
<tr>
<td>Oxidized glutathione (μmol/l)</td>
<td>333.0 ± 124.5</td>
<td>376.5 ± 154.7</td>
<td>43.5</td>
</tr>
<tr>
<td>Reduced/oxidized glutathione</td>
<td>5.0 ± 2.3</td>
<td>6.1 ± 4.3</td>
<td>1.12</td>
</tr>
<tr>
<td>NT (nmol/ml)</td>
<td>16.7 ± 5.3</td>
<td>14.4 ± 4.0</td>
<td>−2.22</td>
</tr>
</tbody>
</table>

$^a$-Values were obtained by comparing differences in the concentrations of the blood variables between the two groups using Student’s t-test, unless otherwise noted.

$^b$Log-transformed values for non-normally distributed values.

$^c$-Values were obtained by comparing differences in the log concentrations of the blood variables between the two groups using Student’s t-test.

doi:10.1371/journal.pcr.0020017.t002

Figure 2. Means and 95% CIs of Between-Group Differences of Changes (in Standardized Units) for the Blood Variables Compared (Experimental Minus Control Group)

For this analysis, all the variables were standardized (mean/overall standard deviation).

doi:10.1371/journal.pcr.0020017.g002
regulated. It could be hypothesized that the raised resistin values occasionally found to be associated with type 2 diabetes or obesity and, more consistently, with endothelial dysfunction and inflammation, rather than being linked to insulin resistance, might be associated with increased oxidative stress, common to all these conditions [41,42].

Limitations
Oxidative stress is not an easily definable condition, and none of the indices used for its evaluation could be defined as the most appropriate criterion in universal terms [43]. Problems of low protocol adherence were not documented. Contamination between the two groups seems unlikely due to the short-term and simple regimen of the intervention.

The open nature of the study and the lack of a placebo might have affected participants’ behavior during the trial. However, it is quite unlikely that this could represent a source of measurement bias, since all the end points (e.g., the relationship between vitamin C and resistin levels) were blood measurements, blindly assessed by the same laboratory.

It is unlikely that dietary patterns would have changed substantially from June–July 2005 to September–November 2005; moreover, all the participants were advised to continue their habitual diet. As participants were randomly allocated in the two groups and were similar for many environmental characteristics (smoking, alcohol and nutrient intake, education level, and physical activity), there is no reason why only one group should have changed dietary habits. Vitamin C levels slightly decreased in the control group, and a seasonal effect might be the most probable cause (higher fruit/vegetable intake is expected during the summer). In the experimental group, this change might not be appreciable, owing to the ascorbic acid supplementation received. The slight reduction in vitamin C plasma levels of the control individuals corresponded to a small increase in their serum resistin values. This unexpected finding is not only consistent with but it also further strengthens the results found in the experimental group.

The loss of two volunteers in the control group, because they moved away, is unlikely to have influenced the main results of the study. An intention-to-treat analysis, assuming for these two control individuals the same average resistin reduction of the experimental group, confirmed the results.

Until further studies confirm these results in other cohorts (patients with diabetes, obesity, etc.) and the potential benefits of antioxidant treatment are demonstrated, no clinical consideration could be extrapolated from our data.

Generalizability
This explicative trial was performed in healthy volunteers, and it may be reasonable to generalize its results to healthy groups. Further studies are needed to test these relationships in individuals with health problems or who are taking medication.

Overall Evidence
Existing evidence on resistin participation in oxidative processes is scarce and heterogeneous; most studies were performed in vitro or in animals [23,24,26]. In humans, a small study on patients with psoriasis showed normalization of the increased resistin serum levels after treatment with retinoid therapy [25]. This is to our knowledge the first trial in humans designed to assess the impact in vivo of antioxidant treatment on resistin serum levels. Its main result is that vitamin C significantly reduced resistin, without

![Figure 3. Before–After Change in Resistin versus Before–After Change of Vitamin C](https://www.plosclinicaltrials.org/article-pdf/00200017/g003)
changing other metabolic/inflammatory parameters. This suggests a specific direct effect of antioxidant substances on resistin expression from monocytes/macrophages.

Conclusion
The exact biological role of resistin in humans remains so far equivocal. These results add some data to currently available knowledge by demonstrating an inverse relationship between blood levels of resistin and vitamin C. Future investigations on resistin participation in oxidative processes are warranted to further clarify the intriguing, but not well-defined role of this protein in chronic degenerative diseases.

SUPPORTING INFORMATION
CONSORT Checklist
Found at doi:10.1371/journal.pctr.0020017.sd001 (41 KB DOC).

Trial Protocol
Found at doi:10.1371/journal.pctr.0020017.sd002 (47 KB DOC).

ACKNOWLEDGMENTS
We are indebted to Dr. Federica Ghione and Dr. Sabrina Guidi, for their valuable assistance in performing the study.

Author Contributions
SB participated in the conception and design of the study, supervision of data collection, data analysis, interpretation of the findings of the study, and manuscript writing and revision. GC participated in the design and randomization of the trial, data analysis, and manuscript writing and revision. MD, RG, PM, and AB participated in data collection, interpretation of the findings, and manuscript revision. ET, DP, and MC participated in data collection and manuscript revision. GP participated in the conception and design of the study, interpretation of the findings of the study, and manuscript writing and revision. All authors have given final approval of the manuscript submitted.

REFERENCES