Vanin 1 Gene Role in Modulation of iNOS/MCP-1/TGF-β1 Signaling Pathway in Obese Diabetic Patients

Yasser O Mosaad1, Mohammed Abdalla Hussein2, Hayam Ateyya3,4, Ahmed H Mohamed5, Ali A Ali6, Alaa Ramadan Youssuf7, Michael Wink8, Amal A El-Kholy9

1Department of Pharmacy, Practice & Clinical Pharmacy, Faculty of Pharmacy, Future University, Cairo, Egypt; 2Department of Biotechnology, Faculty of Applied Health Science, October 6th University, October 6th City, Egypt; 3Department of Medical Pharmacology, Faculty of Medicine, Cairo University, Cairo, Egypt; 4Department of Pharmacy Practice and Clinical Pharmacy, Faculty of Pharmacy, Future University, Cairo, Egypt; 5Department of Radiology and Medical Imaging, Faculty of Applied Health Science Technology, October 6th University, October 6th City, Egypt; 6Food Sciences Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt; 7Consultant and Head of Cardiology Department, AL-AHRAR Teaching Hospital, Zagazig University, Zagazig, Egypt; 8Heidelberg University, Institute of Pharmacy and Molecular Biotechnology, Heidelberg, Germany; 9Department of Clinical Pharmacy, Faculty of Pharmacy, Ain-Shams University, Cairo, Egypt

Correspondence: Mohammed Abdalla Hussein, Faculty of Applied Health Sciences Technology, October 6th University, October 6th City, Egypt, Tel +20 1224832580, Email prof.husseinma@o6u.edu.eg

Introduction
Cysteamine, a powerful endogenous antioxidant, is produced mostly by the vanin-1 with pantetheinase activity. With regard to glycemic, inflammatory, and redox factors, the current study sought to evaluate the association between the expression of the vanin-1 gene, oxidative stress, and inflammatory and iNOS signaling pathway in obese diabetic patients.

Methods: We enrolled 67 male subjects with an average age of 53.5 ± 5.0 years, divided into 4 groups according to the WHO guideline. We determined their plasma levels of glucose, insulin, IRI, HbA1c, TC, TG, HDL-C, TNF-α, MCP-1, TGF-β1, SOD, CAT, and TBARs, as well as expression of the iNOS and Vanin1 genes.

Results: Overweight and obese class I and II diabetics had significantly higher levels of plasma glucose, insulin, HbA1c, TNF-α, MCP-1, TGF-β1, CAT, and TBAR as well as iNOS and vanin-1 gene expression compared to healthy control individuals. In addition, as compared to healthy control individuals, overweight obese class I and II diabetics’ plasma HDL-C levels and blood SOD activity were significantly lower. In addition, ultrasound and computed tomography showed that the presence of a mild obscuring fatty liver with mild hepatic echogenicity appeared in overweight, class I and II obese diabetic patients.

Conclusion: These findings provide important information for understanding the correlation between Vanin 1 and glycemic, inflammatory, and redox factors in obese patients. Furthermore, US and CT analysis were performed to visualize the observed images of fatty liver due to obesity.

Keywords: overweight, BMI, obese diabetic patients, iNOS, vanin-1, MCP-1, US, CT

Introduction
Obesity and insulin resistance (IR) are the components of the metabolic syndrome which is strongly associated with oxidative stress.1 The most important problem in patients with metabolic syndrome is an increased cardiovascular risk. Oxidative stress is also involved in the pathogenesis of cardiovascular disease.2 It is worth mentioning that the metabolic syndrome is associated with an increased arterial stiffness and endothelial dysfunction. Perivascular adipose tissue was shown to take part in the pathogenesis of cardiovascular disease. It is also worth mentioning that there are metabolically healthy obesity persons, but a metabolically healthy obesity can change into the metabolically unhealthy state.3

The establishment of IR is also associated with obesity.4 In contrast to patients with subcutaneous obesity, people with central obesity exhibit a higher rate of insulin resistance.5,6 According to some experts, IR is thought to be the primary...
factor for metabolic and nonmetabolic disorders. Also, Grundy et al reported that the elevated pancreatic insulin production results from peripheral tissue insulin resistance. Hyperinsulinemia provides normal blood glucose levels during this phase. Finally, insulin resistance may outweigh the ability of the pancreas to compensate, and blood glucose levels start to rise.

In addition, elevated BMI shows a negative link with HDL-C as well as a positive correlation with TC, LDL-C, and TG levels. The link between BMI and lipoprotein concentrations, particularly LDL-C, has been found to be a substantial contributor to cardiovascular disease in obese individuals. Inflammatory adipokines such IL-6, TNF-α and MCP-1 are produced when macronutrient intake rises, and this results in chronic inflammation in obese people.

Furthermore, an increase in free radical generation is linked to both diabetes mellitus and obesity. These free radicals can interact with proteins, lipids, DNA, and other biological elements, which would injure cells and cause mutations.

It has been recognized that iNOS development and obesity are related to each other. According to several studies, patients with uncontrolled Type 2 diabetes had iNOS protein levels in skeletal muscle that were nearly four times higher than those of the control group.

Other proteins, such as vanin-1 vascular protein, are associated with obesity, diabetes and cardiovascular diseases. Vanin-1 functions as a pantherinae that catalyzes the degradation of pantetheine to pantothenic acid and releases
cysteamine. Vanin-1 is linked to the membrane through a glycosylphosphatidylinositol (GPI). Cysteamine, a potent antioxidant, is widely produced in the blood, kidneys, and hepatocytes.

Recently, Vanin 1 has also been studied in relation to ulcerative colitis, for instance drug-induced toxicity. DSS (dextran sulfate sodium) is one of the most used drugs, for induction of ulcerative colitis in experimental rats. It can lead to severe necrosis in the tissue of colon. In the current study, we examined the expression of vanin-1 in obese/diabetic patients and its relationship to glycemic, inflammatory, and redox factors.

**Methods**

**Human Participants**

We enrolled 67 participants from the AL-AHRAR Teaching Hospital in Egypt between September 2020 and April 2021 for this prospective non-randomized trial. The 67 male patients had a mean age of 53.5±5.0 years. They were categorized into the following 4 groups based on their BMI according to WHO:

- **Group 1**: control group, 18 healthy men with BMI < 25 kg/m².
- **Group 2**: consisted of 16 overweight diabetic individuals with a BMI of 25 to 29.9 kg/m².
- **Group 3**: comprised 20 class I obese diabetic patients (30.00–34.9 kg/m²).
- **Group 4**: consisted of 13 class II obese diabetic patients with a BMI between 35 and 39.9 kg/m².

The body mass indices of the participants were determined after a thorough assessment of their medical history, using the formula BMI = body weight kg/height (m²). When they were chosen for the study, none of the participants were following a specific diet.

Estimating fasting plasma glucose (FPG) and insulin levels for type 2 diabetes (DM) diagnosis. Using the World Health Organization’s 1998 diagnostic criteria, DM and normal glucose metabolism were identified. FPG 7.0 mmol/L is the threshold for DM.

**Criteria for Exclusion**

Patients with cancer, serious infectious or inflammatory disorders, autoimmune diseases, and serious systemic diseases (liver, kidney, rheumatic, and cardiovascular diseases) were excluded from the study. Patients who had taken antibiotics, anti-inflammatory drugs, or corticosteroids were excluded.

**Blood Sampling and Biochemical Analyses**

Diagnosis of participants was carried out by Prof. Alaa Ramadan Mohamed Youssuf, MD. Cardiology, Consultant and Head of Cardiology Department, AL-AHRAR Teaching Hospital, Zagazig University, Egypt. All experimental procedures were carried out following the ethical standards under a protocol approved by the Ethics Committee of AL-AHRAR Teaching Hospital, Zagazig University, Egypt (HAH00019) and were executed conforming to the Guide for medical research involving human subjects that was initially adopted by the 18th assembly of the World Medical Association in Helsinki, Finland, in June 1964. Venous blood samples (after fasting overnight) were obtained from all participants, anticoagulated, and divided into two parts; the first part was used for the measurement of glycated hemoglobin (HbA1c) using ARCHITECT c 4000 analyzers (Abbott Diagnostics), and the second part was centrifugated and the plasma obtained was used for the colorimetric determination of glucose, TC, TG and HDL-C. In addition, plasma insulin, TNF-α, MCP-1, TGF-β1, SOD, CAT, and TBARs were measured by MaxSignal® HTS DON ELISA Kits (Llantrisant, United Kingdom). Insulin resistance index (IRI) was measured according to the formula of Viktorinova et al. IRI = insulin (mU/mL) x glucose (mM)/22.5.

**qRT-PCR**

Using the RNA-spinTM (Qiagen GmbH, Hilden, Germany), total RNA of plasma was extracted. As instructed by the manufacturer, cDNA was utilized for qPCR using SYBR Green PCR master mix (iNiRON Biotechnology, Korea). The reverse transcription kit was used to produce cDNA from 1 to 5 µg total RNA (Applied Biosystems, Foster City, CA).

The PCR reaction mixture contained 0.4 M of primers, 1.5 mM MgCl₂, 200 µM dNTPs, 0.5 µM of sense and antisense primers, and 2.5 units of Taq DNA polymerase (Promega Corporation). The PCR reaction mixtures were heated for the necessary number of cycles for 45s each at 94°C, for 30s at the proper annealing temperatures, and for one minute and thirty seconds at 72°C. The extension step was then carried out at 72°C for 10 minutes. The housekeeping gene
glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was utilised to normalise the CYP mRNA concentration. Primer sequences of INOS and vanin-1 genes as well as GAPDH are illustrated in Table 1.

### Ultrasound Scanning Procedure

Using the US apparatus with 7 MHZ power linear transducer, the patients underwent an abdominal plain ultrasound procedure (Philips CV650). The patients’ stomachs were shaved to reduce imaging artefacts and to prevent vomiting and gas from the abdomen area during the US scan. Patients were not allowed to eat eight hours prior to the trial as part of this preparation. The probe was positioned longitudinally and transversely in the upper hypochondrium to investigate the liver. This made it possible to capture several longitudinal and transverse images.

### Computed Tomography (CT)

Plain abdominal CT scans were performed in patients (Spiral, Toshiba, Activation 16, Tochigi, Japan) with a scan time of 0.48 s (partial), 0.75–3 s, as well as a row data memory size of 144 GB and a 75 GB image data memory size in the slice thickness with 0.5–5 mm. CT numbers were taken in liver and spleen at three different locations, excluding blood arteries and heterogeneous parts. CT imaging was used to assess the fatty liver of overweight and obese diabetic patients.

### Statistical Analysis

Data were expressed as means ± SD. ANOVA with Spearman’s rank correction (ρ) was used to evaluate all data using the SPSS/20 program. The significant differences between the two groups of overweight, class I and II obese diabetic patients, and control participants were compared using a Student’s t-test. Data at (p < 0.05) are regarded as significant.

### Results

Table 2 documents that the levels of BMI, glucose, insulin, IRI as well as glycated hemoglobin (HbA1c) among healthy males as well as diabetic overweight and obese diabetic patients. BMI values differ significantly (p < 0.05) between four

---

**Table 1 Primers Used in Real-Time PCR**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inducible nitric oxide synthase</td>
<td>F: 5′-CGA GGA GGC TGC CCT GCA GAC TGG-3′</td>
</tr>
<tr>
<td></td>
<td>R: 5′-CTG GGA GGA GCT GAT GGA GTA GTA-3′</td>
</tr>
<tr>
<td>Vanin-1</td>
<td>F: 5′-GGAAACGGGTATGTCTTCCC-3</td>
</tr>
<tr>
<td></td>
<td>R: 5′-ACTCCCCAGGTGCAGG-3</td>
</tr>
<tr>
<td>GAPDH (Internal control for qRT-PCR)</td>
<td>F: 5′- CCA TCACCATCTCCAGG AG-3′</td>
</tr>
<tr>
<td></td>
<td>R: 5′-CTGCTTCCACCACCTTCTTG -3′</td>
</tr>
</tbody>
</table>

---

**Table 2 BMI in Relation to Glucose, Insulin, IRI and HbA1c Among Healthy Persons and Overweight as Well as Class I and II Obese Diabetic Patients**

<table>
<thead>
<tr>
<th>No.</th>
<th>Groups</th>
<th>BMI (kg/m²)</th>
<th>Glucose (mmol/L)</th>
<th>Insulin (μU/mL)</th>
<th>IRI</th>
<th>HbA1c (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>Healthy males (N = 18)</td>
<td>23.25 ± 4.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.41 ± 0.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.77 ± 0.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.39 ± 0.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.67 ± 0.54&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>(2)</td>
<td>Overweight diabetic patients (N = 16)</td>
<td>30.47 ± 6.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.76 ± 0.79&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.51 ± 0.72&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.59 ± 0.39&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.45 ± 1.32&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>(3)</td>
<td>Class I obese diabetic patients (N = 20)</td>
<td>35.20 ± 4.60&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.34 ± 0.84&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.71 ± 1.10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.61 ± 0.57&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.88 ± 1.45&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>(4)</td>
<td>Class II obese diabetic patients (N = 13)</td>
<td>38.10 ± 3.37&lt;sup&gt;d&lt;/sup&gt;</td>
<td>10.63 ± 0.64&lt;sup&gt;d&lt;/sup&gt;</td>
<td>10.54 ± 1.56&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.96 ± 0.73&lt;sup&gt;d&lt;/sup&gt;</td>
<td>10.31 ± 1.10&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

**Notes:** Data shown are mean ± standard deviation of number of observations within each treatment. Data followed by different superscript alphabet along the same column are significantly different (p < 0.05). The high significant levels of the parameters were in the order of a < b < c < d. Data with superscript alphabet <sup>a</sup>Are significantly lower than data with superscript alphabet <sup>b</sup>While data with superscript <sup>c</sup>Are lower than data with superscript alphabet <sup>d</sup>At p < 0.05. Data followed by the same superscript alphabet are not significantly different at p ≤ 0.05. IRI = insulin (μU/mL) x glucose (mM) / 22.5.

**Abbreviations:** BMI, Body mass index; BP, Blood pressure; IRI, Insulin resistance index; HbA1c, glycated hemoglobin; N, number of participants in each group.
groups. Glucose levels were significantly increased by 43.44%, 72.64%, and 96.49% in overweight as well as class I and II obese patients, respectively, in comparison to healthy controls (p < 0.05). In parallel, the levels of glucose, insulin, insulin resistance and glycated hemoglobin were increased in overweight and obese patients: Similarly, plasma insulin was significantly increased by 30.15%, 50.95%, and 82.66% in overweight as well as class I and II obese diabetic patients, respectively, as compared to the healthy group (p < 0.05). Changes in IRI were even more pronounced; its levels increased significantly by 68.18%, 134.41%, and 222.07% in obese and overweight class I and II patients, respectively, in comparison to healthy group (p < 0.05). Furthermore, HbA1c plasma levels were increased non-significantly by 6.43% overweight patients and significantly by 63.03%, and 70.13% in overweight as well as class I and II obese patients, respectively, in comparison to healthy group (p < 0.05).

Plasma lipid parameters, such as TC, TG, and HDL-C among healthy males as well as diabetic overweight and obese diabetic patients are tabulated in Table 3. Plasma TC was increased significantly by 20.48%, 32.90%, and 42.94% in overweight, class I and II obese patients, respectively, in comparison to the healthy control group (p < 0.05). TG was significantly increased by 36.82%, 68.60%, and 83.0% in overweight as well as class I and II obese patients, respectively, compared to controls (p < 0.05). However, the plasma HDL-C levels decreased non-significantly by 10.94% in overweight patients, and significantly by 19.15% (p < 0.05) and 24.73% (p < 0.05) in class I and II obese patients in comparison with healthy control patients.

Table 4 shows the levels of inflammation markers, such as plasma TNF-α, MCP-1 and TGF-β1 among healthy males as well as overweight and obese diabetic patients. Values for TNF-α were increased by 24.50%, 70.54% and 116.08% in overweight comparison with healthy control patients.

**Table 3 Levels of TC, TG and HDL-C Among Healthy Persons, Overweight as Well as Obese Class I and II Diabetic Patients**

<table>
<thead>
<tr>
<th>No.</th>
<th>Groups</th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>Healthy males (N = 18)</td>
<td>186.04 ± 25.22</td>
<td>122.13 ± 20.08</td>
<td>46.04 ± 10.21</td>
</tr>
<tr>
<td>(2)</td>
<td>Overweight diabetic patients (N = 16)</td>
<td>224.15 ± 13.31</td>
<td>167.11 ± 15.12</td>
<td>41.00 ± 4.62</td>
</tr>
<tr>
<td>(3)</td>
<td>Obese Class I obese diabetic patients (N = 20)</td>
<td>247.26 ± 23.79</td>
<td>205.92 ± 25.90</td>
<td>37.22 ± 4.34</td>
</tr>
<tr>
<td>(4)</td>
<td>Class II obese diabetic patients (N = 13)</td>
<td>265.94 ± 18.78</td>
<td>223.51 ± 25.77</td>
<td>34.65 ± 3.71</td>
</tr>
</tbody>
</table>

**Notes:** Data shown are mean ± standard deviation of number of observations within each treatment. Data followed by different superscript alphabet along the same column are significantly different (p < 0.05). The high significant levels of the parameters were in the order of a < b < c < d. Data with superscript alphabet aAre significantly lower than data with superscript alphabet bWhile data with superscript bAre lower than data with superscript alphabet cAt p < 0.05. Data followed by the same superscript alphabet are not significantly different at p ≤ 0.05.

**Abbreviations:** TC, Total cholesterol; TG, triacylglycerols; HDL-C, high-density lipoprotein-cholesterol; N, number of participants in each group.

**Table 4 Levels of Plasma TNF-α, MCP-1 and TGF-β1 in Healthy Persons and Overweight as Well as Obese Class I and II Diabetic Patients**

<table>
<thead>
<tr>
<th>No.</th>
<th>Groups</th>
<th>TNF-α (pg/mL)</th>
<th>MCP-1 (pg/mL)</th>
<th>TGF-β1 (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>Healthy males (N = 18)</td>
<td>4.04 ± 0.40</td>
<td>230.37 ± 18.00</td>
<td>35.55 ± 3.901</td>
</tr>
<tr>
<td>(2)</td>
<td>Overweight diabetic patients (N = 16)</td>
<td>5.03 ± 0.52</td>
<td>263.60 ± 19.93</td>
<td>41.27 ± 4.98</td>
</tr>
<tr>
<td>(3)</td>
<td>Class I obese diabetic patients (N = 20)</td>
<td>6.89 ± 0.64</td>
<td>307.77 ± 27.58</td>
<td>49.25 ± 4.08</td>
</tr>
<tr>
<td>(4)</td>
<td>Class II obese diabetic patients (N = 13)</td>
<td>8.73 ± 1.28</td>
<td>382.23 ± 30.24</td>
<td>54.69 ± 3.34</td>
</tr>
</tbody>
</table>

**Notes:** Data shown are mean ± standard deviation of number of observations within each treatment. Data followed by different superscript alphabet along the same column are significantly different (p < 0.05). The high significant levels of the parameters were in the order of a < b < c < d. Data with superscript alphabet aAre significantly lower than data with superscript alphabet bWhile data with superscript bAre lower than data with superscript alphabet cAt p < 0.05. Data followed by the same superscript alphabet are not significantly different at p ≤ 0.05.

**Abbreviations:** TNF-α, Tumor necrosis factor-α; MCP-1, Monocyte Chemoattractant Protein-1; TGF-β1, Transforming growth factor-beta 1; N, number of participants in each group.
patients, class I and II obese diabetic patients, respectively, as compared to the healthy group of males (p < 0.05). Similarly, plasma MCP-1 levels increased significantly by 14.42%, 33.59%, and 65.92% in overweight, class I and II obese diabetic patients, respectively, as compared to the control (p < 0.05). Finally, the level of plasma TGF-β1 was significantly increased by 16.09%, 38.53%, and 53.83% in overweight, class I and II obese diabetic patients, respectively, in comparison to the healthy group (p < 0.05).

The present study also interested in the antioxidative capacity of the patients. Table 5 shows blood SOD and CAT as well as plasma TBARs levels among healthy males as well as overweight and obese diabetic patients. Blood SOD activity decreased significantly by 13.20%, 24.27% and 32.03% in overweight patients, obese diabetic patients of class I and II, respectively, in comparison to the healthy control group (p < 0.05). Blood CAT activity was significantly increased by 51.05%, 66.63%, and 87.67% in overweight as well as class I and II obese diabetic patients in comparison with the control group (p < 0.001). Similarly, plasma TBARs levels were increased significantly (p < 0.05) by 61.11%, 106.94%, and 186.11% in overweight, class I and II obese diabetic patients, respectively, compared to the control group.

In another set of analyses, the present study was interested in the expression levels of iNOS and vanin-1 gene in plasma cells. Figures 1 and 2 show gene expression of iNOS and vanin-1 among healthy males as well as overweight and obese patients. Plasma iNOS gene expression (Figure 1) was significantly increased by 38.37%, 105.81%, and 227.90% in overweight patients, obese diabetic patients of class I and II, respectively, in comparison to the healthy control group (p < 0.05). Blood CAT activity was significantly increased by 51.05%, 66.63%, and 87.67% in overweight as well as class I and II obese diabetic patients in comparison with the control group (p < 0.001). Similarly, plasma TBARs levels were increased significantly (p < 0.05) by 61.11%, 106.94%, and 186.11% in overweight, class I and II obese diabetic patients, respectively, compared to the control group.

In another set of analyses, the present study was interested in the expression levels of iNOS and vanin-1 gene in plasma cells. Figures 1 and 2 show gene expression of iNOS and vanin-1 among healthy males as well as overweight and obese patients. Plasma iNOS gene expression (Figure 1) was significantly increased by 38.37%, 105.81%, and 227.90% in overweight patients, obese diabetic patients of class I and II, respectively, in comparison to the healthy control group (p < 0.05). Blood CAT activity was significantly increased by 51.05%, 66.63%, and 87.67% in overweight as well as class I and II obese diabetic patients in comparison with the control group (p < 0.001). Similarly, plasma TBARs levels were increased significantly (p < 0.05) by 61.11%, 106.94%, and 186.11% in overweight, class I and II obese diabetic patients, respectively, compared to the control group.

![Figure 1](https://doi.org/10.2147/JIR.S386506)

**Figure 1** iNOS gene expression levels among healthy persons and overweight as well as class I and II obese diabetic patients. Data shown are mean ± standard deviation of number of observations within each treatment. Data followed by different superscript alphabet along the same column are significantly different (p < 0.05). The high significant levels of the parameters were in the order of a < b < c < d. Data with superscript alphabet “a” are significantly lower than data with superscript alphabet “b” while data with superscript “b” are lower than data with superscript alphabet “c and d” at p < 0.05. Data followed by the same superscript alphabet are not significantly different at p ≤ 0.05.

**Abbreviation:** N, number of participants in each group.

### Table 5 Levels of Blood SOD and CAT as Well as Plasma TBARs Among Healthy Persons and Overweight as Well as Class I and II Obese Diabetic Patients

<table>
<thead>
<tr>
<th>No.</th>
<th>Groups</th>
<th>SOD (U.min/mL)</th>
<th>CAT (μ Moles of H₂O₂ Consumed min/mL)</th>
<th>TBARs (μ Mole/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>Healthy males (N = 18)</td>
<td>5.15 ± 0.74⁴</td>
<td>20.92 ± 3.32³</td>
<td>0.72 ± 0.15⁵</td>
</tr>
<tr>
<td>(2)</td>
<td>Overweight diabetic patients (N = 16)</td>
<td>4.47 ± 0.60⁴</td>
<td>31.60 ± 4.12³</td>
<td>1.16 ± 0.20⁶</td>
</tr>
<tr>
<td>(3)</td>
<td>Class I obese diabetic patients (N = 20)</td>
<td>3.90 ± 0.48⁵</td>
<td>34.86 ± 2.8⁵</td>
<td>1.49 ± 3.38⁶</td>
</tr>
<tr>
<td>(4)</td>
<td>Class II obese diabetic patients (N = 13)</td>
<td>3.50 ± 0.29⁶</td>
<td>38.08 ± 4.47⁷</td>
<td>2.06 ± 0.33⁸</td>
</tr>
</tbody>
</table>

**Notes:** Data shown are mean ± standard deviation of number of observations within each treatment. Data followed by different superscript alphabet along the same column are significantly different (p < 0.05). The high significant levels of the parameters were in the order of a < b < c < d. Data with superscript alphabet “a” are significantly lower than data with superscript alphabet “b” while data with superscript “b” are lower than data with superscript alphabet “c and d” at p < 0.05. Data followed by the same superscript alphabet are not significantly different at p ≤ 0.05.

**Abbreviations:** SOD, Superoxide dismutase; CAT, catalase; TBARs, thiobarbituric acid reactive substances; iNOS, inducible NO synthase; N, number of participants in each group.
A correlation analysis between the level of gene expression of vanin-1 and all other variables (studied in Tables 2–5) is documented in Table 6. Significant correlations are apparent in class I and II obese diabetic patients. Furthermore, levels of vanin-1 gene expression are also correlated with iNOS gene expression (r = 0.192, 0.247 and 0.329) and inversely correlated with HDL-C level, and SOD activity (r = −0.265, −0.118 and −0.123; r = −0.031, −0.090 and −0.613), respectively, in overweight patients, and obese diabetic class I & II patients.

In addition to the assessment of biochemical markers, the present study examined the liver of patients by Ultrasound scanning (US) and Unenhanced CT (CT). A normal echo pattern of hepatic parenchyma in healthy males is provided in Figure 2. Vanin-1 gene expression levels among healthy persons and overweight as well as class I and II obese diabetic patients. Data shown are mean ± standard deviation of number of observations within each treatment. Data followed by different superscript alphabet along the same column are significantly different (p < 0.05). The high significant levels of the parameters were in the order of a < b < c < d. Data with superscript alphabet “a” are significantly lower than data with superscript alphabet “b” while data with superscript “b” are lower than data with superscript alphabet “c” and “d” at p < 0.05. Data followed by the same superscript alphabet are not significantly different at p ≤ 0.05. N= number of participants in each group.

Table 6 Correlation Between the Expression of Vanin 1 and Glucose Homeostasis Indices, HbA1c, Lipid Profile, Inflammatory Mediators, and Oxidative Stress Biomarkers in Healthy Persons and Overweight as Well as Class I and II Obese Diabetic Patients

| Parameters | Vanin 1 | |
|------------|---------|------------|------------|------------|
| | Overweight Patients (N = 16) | Class I Obese Diabetic Patients (N = 20) | Class II Obese Diabetic Patients (N = 13) | |
| | r | p | r | p | r | P |
| BMI | 0.203 | 0.450* | 0.025 | 0.916* | 0.056 | 0.856* |
| Glucose | 0.561 | 0.024 | 0.067 | 0.780* | 0.486 | 0.092* |
| Insulin | 0.209 | 0.438 | 0.039 | 0.871* | 0.421 | 0.152* |
| IRI | 0.531 | 0.034 | 0.002 | 0.995* | 0.620* | 0.024* |
| HbA1c | 0.138 | 0.610 | 0.596 | 0.006* | 0.415 | 0.158* |
| TC | 0.335 | 0.205* | 0.233 | 0.323* | 0.047 | 0.880* |
| TG | 0.013 | 0.961* | 0.066 | 0.783* | 0.327 | 0.276* |
| HDL-C | −0.414 | 0.111* | −0.265 | 0.259* | −0.031 | 0.920* |
| TNF-α | 0.234 | 0.383 | 0.149 | 0.529* | 0.537 | 0.059 |
| MCP-1 | 0.134 | 0.620* | 0.355 | 0.124* | −0.090 | 0.770* |
| TGF-β1 | 0.342 | 0.194* | 0.361 | 0.118* | 0.151 | 0.624* |
| iNOS | 0.192 | 0.475* | 0.247 | 0.293* | 0.329 | 0.272* |
| SOD | −0.096 | 0.724* | −0.123 | 0.605* | −0.613 | 0.026* |
| CAT | 0.151 | 0.578* | 0.212 | 0.370* | 0.183 | 0.550* |
| TBARs | 0.114 | 0.675* | 0.176 | 0.459* | 0.337 | 0.261* |

Note: *p significant at 0.05. Abbreviation: N, number of participants in each group.
US of overweight diabetic patients, showed diffusely hepatic echogenicity but portal and diaphragmatic echogenicity are still appreciable (Figure 3B). In class I obese diabetic patients, the US revealed fatty liver with mild hepatic echogenicity (Figure 3C). In addition, US liver examination of class II obese diabetic patients revealed a fatty liver with an echogenic liver that obscured the echogenic walls of portal venous branches (Figure 3D).

In a last set of examinations, the present study was investigated the liver of the patients by unenhanced CT examination of the healthy controls showed a normal hepatic parenchyma (Figure 4A). However, in overweight diabetic patients, the CT showed a diffuse fat accumulation in the liver. In overweight diabetic patients, a liver attenuation (15 HU) is clearly seen. Compared to the liver, the portal vein vessels (v) appear to be hyperattenuating (Figure 4B).

At CT, a focal fat buildup in liver can be observed. In class I obese patients, an axial contrast-enhanced image taken even during the portal venous phase shows hypoattenuating zones of focused fat accumulation next to falciform and venous muscles, as well as in the porta hepatis, without any indication of a mass effect (Figure 4C).

Both US and CT data show that extensive fat deposition occurs with localized sparing. At comparable levels, a longitudinal US image (a) and an axial unenhanced CT image (b) show significant echogenicity and hypoattenuation, indicating a distributed fat deposition in the liver. Focal sparing (fs) is a shaped area with comparative hypoechoogenicity in one direction and hyperattenuating in the other. In class I obese diabetic individuals, the focal fatty pseudolesion has no mass effect on the surrounding vessel (Figure 4D).

**Discussion**

Obesity can be regarded as a chronic health condition. It is characterized by a high BMI that is correlated with elevated levels of glucose, insulin, and insulin resistance index (IRI). Obesity relates to fatty liver, hypertension, hypercholesterolemia, hypertriglyceridemia and an increased risk of death from cardiovascular disease.
The present study confirmed that increased levels of glucose, insulin, and IRI are associated with obese patients with type 2 diabetes. Early intervention strategies such as a healthy diet and physical activity should focus on the senior population to avoid obesity, and type 2 diabetes.\textsuperscript{38,39}

Vanin-1 controls the availability of pantothenic acid\textsuperscript{40} and CoA metabolism\textsuperscript{41} and is crucial for the liver’s lipid metabolism and taurine synthesis (Figure 5). Pantherinae catalyses the degradation of pantetheine to pantothenic acid and releases cysteamine. Pantothenate kinases phosphorylate vitamin B5 to 4-phosphopantothenate during the synthesis of

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{figure4.png}
\caption{CT scan of liver in healthy persons and overweight diabetic as well as class I and II obese patients. Normal appearance of the liver at unenhanced CT (A). CT of overweight diabetic patients showed diffuse fat accumulation in the liver (B). Also, Focal fat accumulation in the liver at CT in class I obese diabetic patients (C). In addition, CT liver examination of class II obese diabetic patients showed multifocal fat accumulation in the liver at CT (D).}
\end{figure}

\textbf{Abbreviations:} v, Vessel; HU, Hounsfield units; fs, Focal Sparing.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure5.png}
\caption{Schematic diagram of the hydrolysis of Pantetheine.}
\end{figure}
CoA; this reaction is regarded as the rate-limiting step.\textsuperscript{42,43} Phosphopantothenate-cysteine ligase produces 4-phosphopantetheine by combining cysteine with phosphopantothenate, giving the CoA molecule the catalytic thiol group, it needs to bind lipids. PPAT converts 4-phosphopantetheine to 3-dephospho-CoA, which is then transformed to CoA by DPCK in a series of phosphorylation cycles.\textsuperscript{44} Through the production of cysteamine and the prevention-free radical-induced damage of islet β-cells and other pancreatic tissues, vanin-1 activity influences glucose, insulin, and IRI levels and regulates the progression of DM.\textsuperscript{45,46} At this condition, vanin-1 expression is induced upon fasting\textsuperscript{47} and it could be a sign of increased PPAR-α activation.\textsuperscript{48}

The present results showed a direct relationship between overweight and obesity with an enhanced expression of the genes for vanin-1 and iNOS, and increased levels of glucose, insulin, as well as IRI. Vanin-1 has become one of the best known PPARα regulated genes.\textsuperscript{49–51}

PPARγ is expressed in adipocytes to control lipid storage and adipocyte development as well as enhance IR. Furthermore, PPARγ agonists increase adiponectin expression, which reduces liver glucose output by activating AMPK,\textsuperscript{52} and therefore can help to improve vanin-1-induced insulin improvement.\textsuperscript{53} Furthermore, upregulation of vanin-1 mRNA related with PPAR has been seen in diabetics and is directly linked to HbA1c and BMI.\textsuperscript{54}

On the other hand, it was discovered that vanin-1 mRNA was elevated in liver steatosis,\textsuperscript{55} that it occurs before lipid buildup and that, in the fatty liver model, it is mediated differently by different kinds of free fatty acids.\textsuperscript{56} The present study suggests that vanin-1 is highly expressed in class I and II of overweight and obese patients compared to healthy males and was positively related to BMI and IRI.

The relationship between HbA1c, glucose, insulin, and IRI in obese diabetic patients has been mentioned in many previous studies.\textsuperscript{57–59} A positive association was reported between HbA1c and glucose and the TC and TG, as well as a negative relation between HbA1c and HDL-C in obese patients.\textsuperscript{60,61}

Type 2 diabetes risk has been linked to low HDL-C and high TC & TG levels.\textsuperscript{62} The present findings showed that type 2 DM patients, who were overweight or obese had higher levels of TC and TG, as well as vanin-1 mRNA expression.

Vanin-1 has a significant impact on PPARα activation which has been related to elevated expression of fatty acid utilization genes and triglyceride particles breakdown by lipoprotein lipase (LPL). Furthermore, it has been associated with fatty acid transport proteins. In addition, activation of acyl CoA oxidase proceed with peroxisomal and mitochondrial β-oxidation.\textsuperscript{62} Apolipoprotein A1,\textsuperscript{63} the main component of HDL-C, and the ATP-binding transporter A1\textsuperscript{64} are expressed more frequently, which contributes to the reverse cholesterol transport system and aids in HDL-C synthesis in humans.

The relationship between inflammation and increased oxidative stress and insulin resistance in obese diabetic patients has been reported in numerous studies.\textsuperscript{65} The present study showed a significant elevation in plasma MCP-1, IL-6, TNF-α, and TGF-β1 among overweight and obese diabetic patients.\textsuperscript{66} The cytokines too stimulate macrophages and monocytes to create reactive oxygen and nitrogen, an elevation in their concentration might be cause of increased oxidative stress. Given how vanin-1 is hyperactivated in response to inflammatory and oxidative stress,\textsuperscript{67,68} the present study suggests the overexpression of vanin-1 in overweight and obese diabetic patients induced by oxidative stress. As discussed before, the endogenous antioxidant cysteamine is produced by vanin-1-mediated catalysis. Also, Naquet et al\textsuperscript{69} have reported a significant decrease in the liver CoA level in obese rats. The present study suggests that the overexpression of vanin-1 in overweight and obese diabetic patients could be the recycling of pantothenic acid and the formation of cysteamine as a potent antioxidant agent (Figure 5). In a model of peripheral inflammation, cysteamine has also shown strong anti-inflammatory effect.\textsuperscript{70} The present observation showed that vanin-1 overexpression is in line with other studies that proved its protection correlated with reduction of apoptosis and inflammation.\textsuperscript{71–73}

The present results demonstrate an excess in blood CAT and TBARs, in addition to a low in blood SOD among overweight and obese diabetic patients. Furthermore, the present results found a positive relationship between BMI and TBARs and CAT, as well as a negative association between BMI and blood levels.

MDA and ROS levels were significantly elevated in high BMI patients.\textsuperscript{74} One significant effect is the modification of the endogenous GSH pool by gamma-glutamylcysteine synthetase,\textsuperscript{75} which affects the redox status and consequently cell fate in response to oxidative stress damage.\textsuperscript{76} Among overweight and obese diabetic patients, elevated CAT activities
point to the presence of oxidative stress. Most peroxisomes include the tetrameric protein CAT. It facilitates the transformation of hydrogen peroxide into water and hydrogen. The increase in CAT could be a compensating strategy for the higher levels of hydrogen peroxide levels. The present findings are consistent with Rindler et al who showed a substantial increase in CAT in fat mice. Indeed, overexpression of CAT is beneficial in preventing oxidative damage.

Rats’ white adipose tissue, which contains iNOS, may be a source of NO generation. We have proven that BMI and vanin-1 gene expression are positively correlated. Furthermore, the present results showed a direct correlation between vanin-1 and iNOS gene expression. We have suggested that vanin-1 overexpression among overweight and obese diabetic patients may be induced by iNOS expression. The correlation between vanin 1 and chronic inflammation is now well understood because of the enzyme’s capacity to generate cysteamine. Vanin 1 also inhibits the expression of the iNOS gene and regulates metabolic pathways in part through the PPAR-α and PPAR-γ.

Additionally, the correlation between iNOS expression and IRI was investigated in many reports. T2D exhibited four times the amount of iNOS expression as normal matched controls. Furthermore, the present findings are consistent with those of Lien et al., Juan et al., and Batra et al. regarding the elevation of iNOS expression and TNF-α resulted in increased lipolysis in this tissue. The present study suggests that a direct correlation between vanin-1 and iNOS expression, inflammatory mediators, and antioxidant enzymes may be due to its role in the production of coenzyme A and cysteamine (Figure 5).

Our study suggests that a direct correlation between vanin-1 and iNOS expression and provides a logical reason to explain the obtained results as follows: in patients who are obese, adipocytes produce fatty acids in tissues in the periphery that express iNOS and TNF-α by preventing lipolysis, vanin-1 helps to maintain normal fatty acid levels. Also, vanin-1 involved in production of Coenzyme A which plays important role in TCA cycle and production of energy. Additionally, vanin-1 contributes to the synthesis of the antioxidant cysteamine, which inhibits hepatic acetyl-CoA carboxylase, hence triggering hepatic fatty acid oxidation.

Conclusion
In the present report, the changes in vanin-1 gene expression in obese/diabetic patients may be related to changes in glycemic, inflammatory, and redox factors. The present study suggests that in obese patients, adipocytes produce fatty acids in tissues in the periphery that express iNOS and TNF-α thus preventing lipolysis. Vanin-1 gene expression in obese/diabetic patients may be related to the elevation of glycemic, inflammatory, and redox parameters and has not been previously documented, and this study may be the first of its kind. More studies are in progress to determine the relationship between vanin-1 and cholesterol biosynthesis and TCA regulating factors.

Abbreviations
BMI, Body mass index; BP, Blood pressure; IRI, Insulin resistance index; HbA1c, glycated hemoglobin; TC, total cholesterol; TG, triacylglycerols; HDL-C, high-density lipoprotein-cholesterol; TNF-α, tumor necrosis factor-α; MCP-1, Monocyte Chemotactant Protein-1; TGF-β1, Transforming growth factor-beta 1; SOD, superoxide dismutase; CAT, catalase; TBARs, thiobarbituric acid reactive substances; iNOS, inducible NO synthase.

Informed Consent Statement
Written informed consent was obtained from all the participants.

Consent for Publication
Consent for publication was received from all the authors.

Author Contributions
All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically

Conclusion
In the present report, the changes in vanin-1 gene expression in obese/diabetic patients may be related to changes in glycemic, inflammatory, and redox factors. The present study suggests that in obese patients, adipocytes produce fatty acids in tissues in the periphery that express iNOS and TNF-α thus preventing lipolysis. Vanin-1 gene expression in obese/diabetic patients may be related to the elevation of glycemic, inflammatory, and redox parameters and has not been previously documented, and this study may be the first of its kind. More studies are in progress to determine the relationship between vanin-1 and cholesterol biosynthesis and TCA regulating factors.

Abbreviations
BMI, Body mass index; BP, Blood pressure; IRI, Insulin resistance index; HbA1c, glycated hemoglobin; TC, total cholesterol; TG, triacylglycerols; HDL-C, high-density lipoprotein-cholesterol; TNF-α, tumor necrosis factor-α; MCP-1, Monocyte Chemotactant Protein-1; TGF-β1, Transforming growth factor-beta 1; SOD, superoxide dismutase; CAT, catalase; TBARs, thiobarbituric acid reactive substances; iNOS, inducible NO synthase.

Informed Consent Statement
Written informed consent was obtained from all the participants.

Consent for Publication
Consent for publication was received from all the authors.

Author Contributions
All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically
reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

**Funding**

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

**Disclosure**

All authors report no conflicts of interest relevant to this article.

**References**


