The effects on oxidative DNA damage of laparoscopic gastric band applications in morbidly obese patients

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Background: Obesity may induce oxidative stress, causing oxidative damage of DNA. We examined associations between decreasing serum and urinary 8-hydroxy-2’-deoxyguanosine (8-OHdG) levels and weight loss in morbidly obese patients before and 6 months after laparoscopic adjustable gastric banding (LAGB).

Methods: We compared patients who had surgery for morbid obesity with healthy, nonobese controls. Urine and fasting blood samples were collected once from the controls and from the morbidly obese patients before and 6 months after the LAGB. The serum and urinary 8-OHdG levels were evaluated in these groups using an enzyme-linked immunosorbent assay kit.

Results: We included 20 patients who had surgery for morbid obesity (8 men, 12 women, mean body mass index [BMI] 46.82 ± 4.47) and 20 healthy, nonobese people (10 men, 10 women, mean BMI 22.52 ± 2.08) in our study. There was no significant difference in serum 8-OHdG levels between the groups, whereas urinary 8-OHdG levels were significantly higher in morbidly obese patients than in controls. Weight, BMI and serum and urinary 8-OHdG levels were significantly decreased in morbidly obese patients 6 months after LAGB.

Conclusion: The LAGB provides efficient weight loss in patients with morbid obesity. The systemic oxidative DNA damage was increased by the morbid obesity, but this increase was not related to weight gain, and it was more evident in serum than urine samples. After LAGB for morbid obesity, the oxidative DNA damage declined both in serum and urine.

Contexte : L’obésité peut provoquer stress oxydatif qui endommage l’ADN. Nous avons analysé les liens entre une baisse des taux de 8-OHdG (8-hydroxy-2’-deoxyguanosine) sériques et urinaires et la perte de poids chez des patients atteints d’obésité morbide avant, puis 6 mois après la pose d’un anneau gastrique ajustable par laparoscopie (AGAL).

Méthodes : Nous avons comparé des patients qui ont subi cette chirurgie pour un problème d’obésité morbide à des témoins non obèses en bonne santé. Nous avons prélevé des échantillons d’urine et de sang à jeun chez les témoins 1 fois et chez les patients atteints d’obésité morbide, avant, puis 6 mois après l’intervention pour AGAL. Les taux de 8-OHdG sériques et urinaires ont été mesurés dans les 2 groupes à l’aide d’une trousse de test ELISA (enzyme-linked immunosorbent assay).

Résultats : Notre étude a inclus 20 patients soumis à la chirurgie pour obésité morbide (8 hommes, 12 femmes; indice de masse corporelle [IMC] moyen 46.82 ± 4.47) et 20 témoins non obèses en bonne santé (10 hommes, 10 femmes; IMC moyen 22.52 ± 2.08). Nous n’avons noté aucune différence significative des taux de 8-OHdG sériques entre les 2 groupes, mais les taux de 8-OHdG urinaires étaient significativement plus élevés chez les patients souffrant d’obésité morbide que chez les témoins. Le poids, l’IMC et les taux de 8-OHdG sériques et urinaires avaient significativement diminué chez les patients atteints d’obésité morbide 6 mois après l’intervention pour AGAL.

Conclusion : L’AGAL est une technique efficace de perte de poids chez les patients souffrant d’obésité morbide. L’atteinte oxydative systémique de l’ADN était exacerbée par l’obésité morbide, mais cette hausse n’était pas reliée au gain pondéral, et elle était plus évidente dans les échantillons sériques que dans les échantillons urinaires. Après la pose d’un AGAL pour obésité morbide, l’atteinte oxydative de l’ADN a diminué dans le sérum et dans l’urine.
Reactive oxygen species (ROS) are produced by living organisms as a result of normal cellular metabolism. At low to moderate concentrations, they function in physiologic cell processes, but at high concentrations, they produce adverse modifications to cell components, such as lipids, proteins and DNA. They cause direct or indirect damage in different organs; thus, it is known that oxidative stress is involved in pathological processes, such as obesity, diabetes, cardiovascular disease and atherogenic processes. It has been reported that obesity may induce systemic oxidative stress. Excess oxidative stress can lead to oxidative damage of DNA, causing significant base damage, strand breaks, altered gene expression and ultimately mutagenesis. 8-hydroxy-2'-deoxyguanosine (8-OHdG) is formed when DNA is oxidatively modified by ROS, and 8-OHdG is one of the most sensitive biomarkers for oxidative stress that can be detected in various biological sample types. Urinary 8-OHdG and its analogues, 8-hydroxyguanosine and 8-hydroxyguanine, are linked to many degenerative diseases.

The prevalence of morbid obesity has increased in developed countries over recent years. Surgical procedures, including laparoscopic adjustable gastric banding (LAGB), Roux-en-Y gastric bypass and vertical banded gastroplasty, have been shown to induce significant and sustained weight reduction. Bariatric surgery is almost the only effective strategy for treating morbidly obese patients. Recent studies found that gastropathy attenuated oxidative stress in obese patients.

We were unable to find data in the literature regarding the effects of LAGB on serum and urinary 8-OHdG levels in morbidly obese patients. The main purpose of this study was to determine the role of LAGB in the modulation of serum and urinary 8-OHdG levels in morbidly obese patients before and 6 months after LAGB. Baseline levels of 8-OHdG in morbidly obese patients were also compared with those in age-matched, healthy, nonobese controls.

Methods

Study population

We consecutively recruited morbidly obese adults from among patients scheduled for LAGB to participate in the study. We also recruited age-matched, nonobese controls from among our clinic staff who did not have an associated illness or any other illness. Surgical therapy should be offered to severely obese patients. The LAGB procedure is a restrictive bariatric method. Patients are eligible for bariatric surgery if they have not responded to nonsurgical treatments and have a body mass index (BMI) above 40 or a BMI above 35 with comorbidities. To be included in the study, patients had to be normotensive, medication-free and have no family history of diabetes or coronary artery disease. The study was approved by the Human Research Ethics Committee of Istanbul University, Cerrahpasa Medical Faculty. We obtained written informed consent from all participants.

Collection of samples

Fasting blood samples were collected in anticoagulant-free tubes once from the nonobese controls and from the morbidly obese patients before and 6 months after LAGB. Immediate centrifugation (3000g) was performed for 15 minutes at 4°C, and the serum was kept at −40°C until analysis. Using sterile containers, we also collected 5 mL of urine from each participant to measure 8-OHdG. Urine samples were centrifuged at 3000g for 10 minutes, before use in the assay, and the supernatants were kept at −80°C until analysis.

Serum and urinary 8-OHdG assay

We determined the concentration of 8-OHdG in the collected samples using a competitive enzyme-linked immunosorbent assay kit (Bioxytech 8-OHdG-EIA Kit, catalogue number 21026, Oxis Health Products, Inc.). The monoclonal antibody in this assay kit recognizes 8-OHdG specifically according to the manufacturer. We used urine creatinine values to correct the daily excretion. Urinary creatinine was measured using the Jeffe reaction. We adjusted the 8-OHdG levels for urinary creatinine levels. Concentration of urinary 8-OHdG was calculated as nanograms per milligram of creatinine. The intra- and interassay coefficients of variation were 6.8% (n = 10) and 7.1% (n = 10), respectively.

Routine serum analysis

Routine serum analyses were determined on an Olympus AU 800 analyzer using enzymatic methods and commercial kits (Roche Diagnostics).

Statistical analysis

Statistical analyses were performed using SPSS software version 20.0 for Windows. We compared the parameters between the morbidly obese group (pre- and postoperative levels) and the control group using the independent-samples t test, and we compared the pre- and postoperative values of the morbidly obese group using the paired t test. The correlation between the parameters was assessed using the Pearson correlation test. We considered results to be significant at p < 0.05.

Results

We included 20 morbidly obese patients (8 men and 12 women, mean age 44.25 ± 12.04 yr, mean BMI 46.82 ±
4.47) and 20 healthy, nonobese controls (10 men and 10 women, mean age 40.70 ± 12.38 yr, mean BMI 22.52 ± 2.08) in our study. The principal anthropometric and biochemical data of the morbidly obese and control groups are summarized in Table 1. There was no significant difference in age or sex between the groups. Body weight and BMI were higher in morbidly obese patients than in healthy, nonobese controls. There was no significant difference in serum 8-OHdG levels between the groups, whereas urinary 8-OHdG levels were significantly higher in morbidly obese patients than in controls (p = 0.014).

Body weight, BMI, serum and urinary 8-OHdG levels were significantly decreased in morbidly obese patients 6 months after gastric banding (p < 0.001). Descriptive characteristics of the morbidly obese patients at baseline and 6 months after the operation are reported in Table 2.

There were no correlations between pre- or postoperative serum 8-OHdG levels and the corresponding urine values. Pre- and postoperative serum and urinary 8-OHdG levels did not correlate with their corresponding weight and BMI.

**DISCUSSION**

The major finding of our study is that serum and urinary 8-OHdG levels, as a systemic oxidative DNA damage marker, in morbidly obese patients decreased independently from weight loss after LAGB for 6 months. The mechanism by which weight loss after LAGB induces oxidative DNA damage is not clear. Decreased 8-OHdG levels may be linked to LAGB through the mechanism of ROS production. If LAGB induces an increase in capacity of 8-OHdG repair enzymes and the downregulation of 8-OHdG production, this may lead to lower levels of oxidative stress. Further studies are required to clarify the involvement and the mechanism of 8-OHdG production in obesity and to clarify the effects of LAGB.

Many studies have shown that obesity is associated with oxidative stress. Formation of 8-OHdG is regarded as a useful indicator of oxygen radical–induced DNA damage. Intracellular accumulation of this compound has been demonstrated in obese patients with diabetes and in patients with human metabolic syndrome, infertility and cancer. Isoprostanes and malondialdehydes, such as lipid (arachidonic acid) peroxidation metabolites, have been reported to be some of the best markers of oxidative damage. However, we used serum and urinary 8-OHdG as the marker of oxidative DNA damage in this study because we focused on the systemic oxidative DNA damage markers rather than general oxidative damage and because we examined protein oxidation, lipid peroxidation and antioxidants in morbidly obese patients in previous studies. All our previous data and other reports collectively support the use of oxidative stress as a significant risk predictor in morbidly obese patients. We selected serum and urinary 8-OHdG levels as markers of oxidative DNA damage in this study because of their convenient use in clinical practice and because they allow the assay to be

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**Table 1. Demographic characteristics and serum and urinary 8-OHdG levels in controls and morbidly obese patients undergoing laparoscopic adjustable gastric banding (baseline)**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group; mean ± SD*</th>
<th>Controls, n = 20</th>
<th>Morbidly obese, n = 20</th>
<th>p value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, male:female</td>
<td></td>
<td>10:10</td>
<td>8:12</td>
<td>0.53</td>
</tr>
<tr>
<td>Age, yr</td>
<td></td>
<td>40.70 ± 12.38</td>
<td>44.25 ± 12.04</td>
<td>0.36</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td></td>
<td>65.55 ± 9.86</td>
<td>132.40 ± 16.28</td>
<td>0.001</td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td>22.52 ± 2.08</td>
<td>46.82 ± 4.47</td>
<td>0.001</td>
</tr>
<tr>
<td>Serum 8-OHdG, ng/mL</td>
<td></td>
<td>1.86 ± 1.92</td>
<td>2.10 ± 2.32</td>
<td>0.55</td>
</tr>
<tr>
<td>Urinary 8-OHdG, ng/mg of creatinine</td>
<td></td>
<td>7.84 ± 7.04</td>
<td>21.02 ± 28.84</td>
<td>0.014</td>
</tr>
</tbody>
</table>

*8-OHdG = 8-hydroxy-2′-deoxyguanosine; BMI = body mass index; SD = standard deviation.
†Statistical difference between morbidly obese versus controls.
‡p < 0.05 statistical significance

**Table 2. Serum and urinary 8-OHdG levels in morbidly obese patients at baseline (preoperative) and 6 months after laparoscopic adjustable gastric banding (postoperative)**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group; mean ± SD</th>
<th>Preoperative, n = 20</th>
<th>Postoperative, n = 20</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, kg</td>
<td></td>
<td>132.40 ± 16.28</td>
<td>107.20 ± 16.47</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td>46.82 ± 4.47</td>
<td>37.89 ± 4.84</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Serum 8-OHdG, ng/mL</td>
<td></td>
<td>2.10 ± 2.32</td>
<td>1.18 ± 0.30</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Urinary 8-OHdG, ng/mg of creatinine</td>
<td></td>
<td>21.02 ± 28.84</td>
<td>8.70 ± 8.12</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

*8-OHdG = 8-hydroxy-2′-deoxyguanosine; BMI = body mass index; SD = standard deviation.
noninvasive. There was no significant difference with regard to serum 8-OHdG levels between the groups, whereas urinary 8-OHdG levels were significantly higher in morbidly obese patients than in controls. In our opinion, this fact should be verified at the next stage of study, using high-performance liquid chromatography as an alternative method for 8-OHdG measurement.

In overweight individuals, an increased BMI has been shown to be associated with increased risk of DNA damage due to oxidative stress. The mechanism by which weight loss induces oxidative DNA damage is not clear. There is controversy surrounding the association between serum 8-OHdG and BMI. Some studies have reported a negative correlation between them, whereas our study found no such correlation. Al-Aubaidy and Jelinek found a positive correlation between 8-OHdG and BMI. Many studies have found a negative correlation between urinary 8-OHdG excretion and BMI. However, we did not find such an association in our study, possibly because of the wide distribution of urinary 8-OHdG levels or our small sample size. Systemic oxidative DNA damage was greater in morbidly obese patients, but this increase was not related to weight gain and was more evident in serum than urine samples. Urinary excretion of 8-OHdG is believed to reflect a general average risk of promutagenic oxidative adducts in the DNA of all tissues and organs.

Obesity is a major contributor to several metabolic disturbances related to oxidative balance. As noted earlier, physical activity or exercise, dietary restriction and surgical interventions reduce oxidative stress levels. Other options for reducing oxidative stress in obese patients include antioxidant therapy. Bariatric surgery is the only effective treatment for treating morbidly obese patients because it affects not only body weight, but also BMI and serum and urinary 8-OHdG levels. Our results suggest that DNA damage caused by ROS occurs more frequently in morbidly obese patients than in age-matched, healthy, non-obese controls and that LAGB reduces DNA damage in morbidly obese patients. We frequently observed increased oxidative DNA damage in morbidly obese patients before surgery and decreased serum and urinary levels of 8-OHdG after LAGB, suggesting that oxidative DNA damage has a powerful association with obesity. Urinary 8-OHdG levels may be useful markers because they can be measured rapidly, easily and cost-effectively. Thus, we recommend that urinary 8-OHdG levels should be more widely assessed in clinical practice. The potential role of 8-OHdG as being responsible for the weight loss after bariatric surgery needs to be elucidated in further studies.

**Limitations**

The present study has several limitations. First, ideally, we would have included an additional group of participants who would have followed the same type of diet without undergoing LAGB. However, owing to physical conditions and material difficulties, we were unable to buy additional kits for additional study groups. In addition, because of the small number of participants, we did not compare the groups according to comorbidities; thus, further studies should explore this issue.

**Conclusion**

Laparoscopic gastric banding is an effective therapeutic approach for treating morbidly obese patients because it affects not only body weight, but also BMI and serum and urinary 8-OHdG levels. Our results suggest that DNA damage caused by ROS occurs more frequently in morbidly obese patients than in age-matched, healthy, non-obese controls and that LAGB reduces DNA damage in morbidly obese patients. We frequently observed increased oxidative DNA damage in morbidly obese patients before surgery and decreased serum and urinary levels of 8-OHdG after LAGB, suggesting that oxidative DNA damage has a powerful association with obesity. Urinary 8-OHdG levels may be useful markers because they can be measured rapidly, easily and cost-effectively. Thus, we recommend that urinary 8-OHdG levels should be more widely assessed in clinical practice. The potential role of 8-OHdG as being responsible for the weight loss after bariatric surgery needs to be elucidated in further studies.

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**Competing interests:** None declared.

**Contributors:** A. Kocael, K. Zengin, M. Taskin, Y. Ersan and H. Uzun designed the study. A. Kocael, H. Erman, G.G. Korkmaz, Y. Ersan and H. Uzun acquired and analyzed the data. R. Gelisgen also acquired the data, and K. Zengin and P.C.A. Kocael also analyzed the data. A. Kocael, P.C.A. Kocael, G.G. Korkmaz, Y. Ersan and H. Uzun wrote the article, which all authors reviewed and approved for publication.
References


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