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# Evaluation of antioxidant systems (coenzyme Q10 and total antioxidant capacity) in morbid obesity before and after biliopancreatic diversion

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**Abstract**

Biliopancreatic diversion (BPD) is a surgical procedure performed in patients with untreatable obesity and insulin resistance. The demonstrated metabolic and hormonal results of this procedure include the reversal of insulin resistance; an increase in diet-induced thermogenesis; and modifications of gut hormones, such as gastrin, enteroglucagon, neurotensin, and cholecystokinin. On the other hand, obesity is a condition of increased oxidative stress; however, few studies have investigated antioxidant systems in obese persons with BPD. To evaluate the metabolic status and antioxidant systems in such patients, we studied a group of 11 morbidly obese patients, aged 28 to 62years, with a mean body mass index (BMI) of 54.71 ± 2.52 kg/m$^2$, before and after successful BPD (mean post-BPD BMI, 44.68 ± 1.51 kg/m$^2$). A control group composed of 10 slightly overweight women, with a mean BMI of 28.5 ± 0.72 kg/m$^2$, was also studied. Coenzyme $Q_{10}$ ($CoQ_{10}$) levels (also normalized for cholesterol levels) and total antioxidant capacity in blood plasma were assessed in these populations. The most striking datum was the extremely low level of $CoQ_{10}$ in postoperative period (0.34 ± 0.16 vs 0.66 ± 0.09 $\mu$g/mL, $P$ = .04); also, the data corrected for cholesterol levels presented the same pattern, with a more marked significance (152.46 ± 11.13 vs 186.4 ± 17.98 nmol/mmol, $P$ = .001).This could be due to lipid malabsorption after surgery. In fact, the pre-BPD data present all the metabolic and hormonal characteristics of severe obesity; and after BPD, there was a net improvement in the metabolic parameters. The first pathophysiologic phenomenon seems to be lipid malabsorption that has been argued to be the cause of insulin resistance reversion. This metabolic interpretation is also confirmed by the absence of significant variations of total antioxidant capacity (57.5 ± 5.3 vs 66 ± 5.3). The mechanisms of these phenomena remain to be established. These data suggest the importance of correcting postsurgical metabolic complications, in these clinical populations, with $CoQ_{10}$ supplementation.


## 1. Introduction

Obesity is considered a principal causative factor in the development of metabolic syndrome and may play a critical role in the pathogenesis of obesity-related comorbidities [1]. The clustering of insulin resistance, hyperinsulinemia, glucose intolerance, hypertension, dyslipidemia, and central obesity constitutes the metabolic syndrome, which is now recognized as a powerful determinant of cardiovascular disease and type 2 diabetes mellitus. The International Diabetes Federation emphasized the need for identification and treatment of the metabolic syndrome because of its strong association with cardiovascular disease [2,3]. The evidence that obesity is related to an increase of oxidative stress has been highlighted [4-7], and an increased plasma level of oxidized low-density lipoprotein in obese people with insulin resistance has been demonstrated [8]; but few studies have comprehensively examined antioxidant systems in human obesity, including coenzyme $Q_{10}$ ($CoQ_{10}$), a lipophilic antioxidant that plays a key part in mitochondrial

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bioenergetics. This molecule may have potential relevance in the metabolic syndrome because of its essential role in oxidative phosphorylation. Moreover, it has tissue-protective and antioxidant properties [9-11], particularly in its reduced state ($CoQ_{10}H_2$). Coenzyme $Q_{10}$ seems to exhibit a unique pattern in the blood plasma: in obese children, who have low levels of lipophilic antioxidants such as α-tocopherol and β-carotene [12,13], $CoQ_{10}$ values are increased [14]. However, this difference disappears when $CoQ_{10}$ concentration is normalized for cholesterol values. In obese adult patients with metabolic syndrome, increased plasma total $CoQ_{10}$ and ubiquinol concentrations were described [15], perhaps as compensation to increased metabolic stress [16]. In our preliminary evaluation, $CoQ_{10}$ plasma levels were markedly reduced by surgically induced weight loss [17], although more data are needed to assess its role in the metabolic syndrome.

Moreover, a recent study investigated plasma total antioxidant capacity (TAC) in obese subjects before and after surgical procedure [18]: oxidative stress related to obesity can be reduced by weight loss obtained either by diet and physical exercise [19,20] or by positioning an intragastric balloon [18]. Various prooxidants with different reactivities and selectivities toward substrates are probably involved in oxidative stress in vivo, and the efficacy of antioxidants depends on the type of oxidants involved. Moreover, in vivo, a particular antioxidant exerts its antioxidant effect together with other antioxidants. Total antioxidant capacity of human plasma might provide valid information in assessing an oxidative stress condition [21].

Biliopancreatic diversion (BPD) is a surgical procedure, performed in patients affected by untreatable obesity and insulin resistance, consisting in a partial gastrectomy with Roux-en-Y reconstruction that results in partial malabsorption and endocrine adaptative modifications including a correction of insulin resistance in terms of insulinemic response to oral glucose tolerance test [22-25]. This surgical procedure might alter lipid absorption and thereby antioxidant absorption.

To evaluate the metabolic status in morbidly obese patients and verify modifications induced by surgical reversal of insulin resistance, we studied $CoQ_{10}$ levels (also normalized for cholesterol levels) and plasma TAC before and after BPD.

## 2. Methods

### 2.1. Patients

Eleven subjects (10 women and 1 man), aged 28 to 62 years and with a mean body mass index (BMI) of 54.71 ± 2.52 kg/m$^2$, entered this study, which was conducted in accordance with the guidelines in the Declaration of Helsinki, after they had given informed consent. They were morbidly obese patients who underwent therapeutic BPD (pre-BPD subjects). They were tested a week before surgical treatment and in a postoperative study, performed 3 to 6 months after successful BPD (post-BPD subjects), when they were in a phase of stabilized BW. Exclusion criteria for our study were conditions with well-known decreased levels of $CoQ_{10}$: cardiac, metabolic, cerebral, neuromuscular, and mitochondrial diseases [26-30].

We also evaluated a control group, composed of 10 slightly overweight women, aged 30 to 45 years, with a mean BMI of 28.5 ± 0.72 kg/m$^2$, chosen to be age-matched with postoperative obese subjects.

### 2.2. Procedures

In the BPD technique, resected gastric volumes range from 200 to 400 mL. The lengths of alimentary and common tracts are 200 and 50 cm, respectively. As a consequence, nutrition does not undergo the normal action of biliary and pancreatic secretions in the alimentary tract. The patients, therefore, develop fat malabsorption (75% of ingested) and a partial starch malabsorption, while maintaining normal absorption of mono- and disaccharides (19% of ingested starch plus mono- and disaccharides); protein absorption remains normal as well. Despite these patterns, calorimetric and body composition analyses demonstrate no signs of malnutrition, as evident by preservation of lean body mass [22,23]. The demonstrated metabolic and hormonal achievements [22,24,25] include (1) the reversal of insulin resistance; (2) an increase in diet-induced thermogenesis; and (3) modifications of gut hormones, such as gastrin, enteroglucagon, neurotensin, and cholecystokinin.

In all patients and controls, a blood sample was collected at 8:00 AM. After centrifugation at 2000 g for 10 minutes, plasma aliquots were immediately stored at −80°C until assayed. We determined basal samples of insulin, glucose, total cholesterol, high-density lipoprotein cholesterol, triglycerides, uric acid, total proteins, $CoQ_{10}$, and TAC in blood plasma.

### 2.3. Blood analyses

Insulin was assayed by radioimmunoassay using kits from Abbott Diagnostics (Milan, Italy); intra- and interassay coefficients of variation were, respectively, 4.5% and 5.6%. Moreover, we determined insulin resistance measured by homeostasis model assessment (HOMA), calculated as basal glucose (in millimoles per liter) × basal insulin (in milli–international units per liter)/22.5, where high HOMA scores (≥2.5) denote low insulin sensitivity (insulin resistance) [31-33].

The $CoQ_{10}$ levels were measured by a well-recognized high-performance liquid chromatography (HPLC) method [34]. The method is based on oxidation of $CoQ_{10}$ in the sample by treating it with *para*-benzoquinone followed by extraction with 1-propanol and direct injection into the HPLC apparatus. Preoxidation of the sample ensures quantification of total $CoQ_{10}$ by UV detection. This method

achieves a linear detector response for peak area measurements over the concentration range of 0.05 to 3.47 μmol/L. Diode array analysis of the peak was consistent with $CoQ_{10}$ spectrum. Supplementation of the samples with known amounts of $CoQ_{10}$ yielded a quantitative recovery of 96% to 98.5%; the method showed a level of quantitation of 1.23 nmol per HPLC injection (200 μL of propanol extract containing 33.3 μL of plasma). A good correlation was found with a reference electrochemical detection method ($r = 0.99$, $P < .0001$). Within run precision showed a coefficient of variation of 1.6 for samples approaching normal values (1.02 μmol/L). Day-to-day precision was also close to 2%.

Reference values of $CoQ_{10}$ are 0.7 to 1 μg/mL [35]. Moreover, $CoQ_{10}$ values were related to plasma cholesterol concentration, measured by a cholesterol-oxidase enzymatic test.

Total antioxidant capacity was evaluated as previously described [36], with a modification of the method developed by Rice-Evans and Miller [37]. The method is based on inhibition, determined by antioxidants, of the absorbance of the radical cation $2,2^{I}$-azinobis(3-ethylbenzothiazoline-6 sulphonate) ($ABTS^{\cdot+}$) formed by interaction between ABTS (150 μmol/L) and ferrylmyoglobin radical species, generated by activation of metmyoglobin (2.5 μmol/L) with $H_2O_2$ (75 μmol/L). Aliquots of the frozen plasma were thawed at room temperature, and 10 μL of the samples was tested immediately. The manual procedure was used with only minor modifications, that is, temperature at 37°C to achieve a more physiologic condition; and each sample was assayed alone to carefully control timing and temperature. The reaction was started directly in cuvette through $H_2O_2$ addition after 1-minute equilibration of all other reagents (temperature control by a thermocouple probe, model 1408 K thermocouple; Digitron Instrumentation, Scunthorpe, United Kingdom) and followed for 10 minutes under continuous stirring, with monitoring at 734 nm, typical of the spectroscopically detectable $ABTS^{\cdot+}$. The presence of chain-breaking antioxidants induces a lag time (the "lag phase") in the accumulation of $ABTS^{\cdot+}$ whose duration is proportional to the concentration of this type of antioxidants. Antioxidant capacity afforded by chain-breaking antioxidants is expressed as the length of lag phase (in seconds). Trolox, a water-soluble tocopherol analog, was used as a reference standard. Standard curves were obtained by using increasing concentrations of Trolox in the same volume as for the sample. Thus, the antioxidant activity can be defined in terms of the concentration of Trolox solution with an equivalent antioxidant potential and expressed as Trolox equivalent antioxidant capacity (TEAC) values (in millimoles per liter). Absorbance was measured with a Hewlett-Packard 8450A UV/Visible spectrophotometer (Palo Alto, CA) equipped with a cuvette stirring apparatus and a constant temperature cell holder. Measurements of pH were made with a PHM84 Research pH meter (Radiometer, Copenhagen, Denmark); the electrode response was corrected for temperature. Unless stated differently, experiments were repeated 2 to 3 times; qualitatively similar results were obtained with individual values varying by less than 8%.

In the lag mode, the assay mainly measures nonprotein and nonenzymatic antioxidants that are primarily extracellular chain-breaking antioxidants, such as ascorbate, urate, and glutathione [37].

### 2.4. Statistics

All results are expressed as the mean ± SEM. Distribution of data was estimated by the test of Kolmogorov-Smirnov. Because the data were nonnormally distributed, the comparison among groups was made using Mann-Whitney $U$ test; the comparison in the same patients before and after BPD was performed using Wilcoxon rank sum test. The Software Arcus Quickstat (Biomedical Version 1.2; Software Publishing, Cambridge, UK) was used for this statistical analysis.

## 3. Results

### 3.1. Clinical and metabolic data

Clinical data and metabolic parameters in our patients and controls are shown in Table 1. As expected, we observed a significant decrease of BMI, insulin levels, HOMA index, uric acid, and total proteins when comparing preoperative and postoperative (3-6 months) evaluations.

### 3.2. Antioxidant data

The $CoQ_{10}$, $CoQ_{10}$ to cholesterol ratio, and TAC in our patients and controls are reported in Fig. 1. The $CoQ_{10}$ levels (mean ± SEM) were not significantly different in obese, before surgery, vs control subjects (0.66 ± 0.09 and 0.68 ± 0.04 μg/mL, respectively). However, they were significantly decreased after surgery (0.34 ± 0.16 μg/mL); the same pattern was observed when considering $CoQ_{10}$ to cholesterol ratio (186.4 ± 17.98 in pre-BPD, 152.46 ± 11.13 in post-BPD, 217.15 ± 20.32 in controls). No significant difference was noted when comparing lag before and after surgery and with control subjects (66 ±

Table 1
Mean ± SEM level of clinical and metabolic parameters in pre- and post-BPD patients and in the control group

| | Pre-BPD | Post-BPD | Controls |
|---|---|---|---|
| BMI (kg/m$^2$) | 54.71 ± 2.52 | 44.68 ± 1.51* | 28.5 ± 0.72 |
| Basal glucose (mg/dL) | 146.91 ± 22.61 | 101.28 ± 0.75 | 92 ± 6.9 |
| Basal insulin (μUI/mL) | 41.47 ± 4.07 | 15.68 ± 1.35* | 16.36 ± 2.86 |
| HOMA index | 11.76 ± 1.06 | 4.14 ± 0.43* | 3.71 ± 0.04 |
| Total cholesterol (mg/dL) | 189.25 ± 8.62 | 153.57 ± 6.61 | 244 ± 7.83 |
| HDL-C (mg/dL) | 43 ± 2 | 45 ± 3.2 | 48.5 ± 2.55 |
| Triglycerides (mg/dL) | 151 ± 21.76 | 118.55 ± 6.12* | 153.5 ± 20.26 |
| Uric acid (mg/dL) | 6.8 ± 0.74 | 3.9 ± 0.24* | 6.35 ± 0.42 |
| Total proteins (g/dL) | 7.53 ± 0.16 | 6.86 ± 0.3* | 7.55 ± 0.12 |

HDL-C indicates high-density lipoprotein cholesterol.
* $P < .05$ vs pre-BPD.

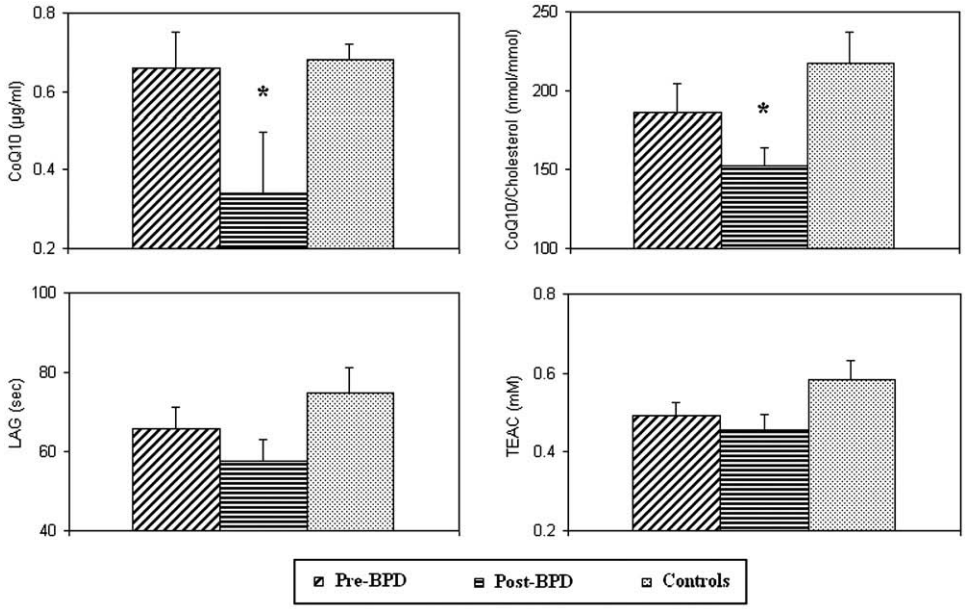


Fig. 1. Levels of $CoQ_{10}$, $CoQ_{10}$ to cholesterol ratio, and TAC (expressed as lag time and TEAC) in our patients, pre- and post-BPD, and in the control group. *$P <$ .05 vs pre-BPD and control group.

5.3, 57.5 ± 5.3, and 75 ± 6.2 seconds, respectively), even if a nonsignificant trend toward lower lag values was present after BPD. Clearly, also, TEAC values (Fig. 1) were not significantly different between pre- and postoperative patients, and vs controls (0.494 ± 0.032, 0.458 ± 0.038, and 0.585 ± 0.049, respectively).

## 4. Discussion

Evidence that obesity is related to an increase in oxidative stress can be found in the literature [4-7]. Increased oxidative stress in a situation of fat accumulation is an important pathogenic mechanism of obesity-associated metabolic syndrome. Fat accumulation is correlated with systemic oxidative stress in humans and mice [4,6,38-43].

The $CoQ_{10}$ has been previously investigated in obese patients with metabolic syndrome [15]: the presence of metabolic syndrome components is associated with increased plasma total $CoQ_{10}$ and ubiquinol concentrations after adjusting for age, sex, and race. This was interpreted as a compensatory mechanism to obesity-induced oxidative stress. Moreover, an increased $CoQ_{10}$ redox ratio (ubiquinol to uniquinone) was reported in women with high-risk metabolic syndrome in the same study. Increased plasma total $CoQ_{10}$ levels were also found in childhood obesity; but after adjustment to plasma cholesterol, no significant difference in $CoQ_{10}$ levels between obese and normal-weight children could be demonstrated [14]. Moreover, the authors did not find $CoQ_{10}$ differences between insulin-resistant and non–insulin-resistant obese children; again, they hypothesized an augmented endogenous $CoQ_{10}$ synthesis to compensate the greater demand of lipophilic antioxidant. In contrast, a decrease was shown in α-tocopherol and β-carotene [12]. Our data showed $CoQ_{10}$ levels similar to those observed in controls, despite the increased oxidative stress. In fact, we reported $CoQ_{10}$ levels in other conditions characterized by oxidative stress, such as hypoadrenalism [44] or hypogonadism [45].

In our preliminary data, $CoQ_{10}$ levels were markedly reduced by the BPD [17]. Accordingly, the most striking data in the present work are the extremely low levels of $CoQ_{10}$ in the postoperative period; this could be due to lipid malabsorption after surgery.

In fact, pre-BPD data present all the metabolic and hormonal characteristics of severe obesity (reduced growth hormone [GH] response to GH-releasing hormone, reduced insulin-like growth factor–1 [IGF-1] and IGF binding protein–3 [IGFBP-3] levels, increased IGF-I/IGFBP-3 ratio, index of increased sensitivity to GH, increase in insulin and HOMA score, increase in leptin levels, and increase in free fatty acid [FFA] levels) [46,47]. After BPD, there was a significant reduction in body weight, BMI, fat mass, and lean body mass; furthermore, a decrease in leptin, insulin, and FFA levels as well as in the HOMA score was observed. In contrast, there was a significant increase in the fasting GH response to GH-releasing hormone, whereas IGF-I and IGF-BP3 remained unchanged. Those data showed a significant positive correlation between insulin and leptin levels as well as a strong negative correlation between insulin and GH secretion pattern [48,49]. During the post-BPD period, there is a net improvement in the metabolic parameters including normalization of insulin levels at oral glucose tolerance test, mainly caused by a decrease in FFA levels [50], in turn determined by lipid malabsorption, as well as decrease in leptin levels, as described in previous

studies [51]. Therefore, the first pathophysiologic phenomenon that has been argued to be the cause of insulin resistance reversion seems to be lipid malabsorption [50]. The ensuing changes in insulin levels, occurring after BPD, constitute the basis for variations in both leptin levels and the somatotrope axis, as previously reported [49,51].

This metabolic interpretation is also confirmed by the absence of significant variations of TEAC and lag, even if a trend toward lower lag values in post-BPD was observed; this could be at least in part explained by the significant decrease of uric acid, which is an important contributor to TAC. A recent study investigated plasma TAC in obese subjects before and after surgical procedure [18]: oxidative stress related to obesity can be reduced by weight loss, obtained either by diet and physical exercise, both in experimental animals and humans [19,20], or by positioning an intragastric balloon [18]. The discrepancy of our data can be explained by the fact that our model is different from other surgically induced weight losses because of its profound effects on fuel absorption and metabolism. Whether $CoQ_{10}$ reduction itself exerts negative effects on metabolism requires further investigations.

In conclusion, our article demonstrated a marked reduction in $CoQ_{10}$ levels after BPD-induced weight loss, without a parallel decrease in TAC. The mechanisms of these phenomena remain to be established. The significant decrease in plasma $CoQ_{10}$ levels might depend on reduced absorption of $CoQ_{10}$ present in the diet or of cofactors and precursors needed for its biosynthesis [52]. These data suggest the importance of correcting post-surgical metabolic complications in these clinical populations with $CoQ_{10}$ supplementation.

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