

## PAPER

# Relationship between sympathetic reactivity and body weight loss in morbidly obese subjects

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**OBJECTIVE:** To investigate the possible role of peripheral sympathetic activity in gastric bypass-induced body weight loss.

**SUBJECTS AND METHODS:** In 42 morbidly obese patients (sex: 36 f/6 m; BMI:  $46.0 \pm 0.7$  kg/m<sup>2</sup>) undergoing a gastric bypass, the skin vasoconstrictor reflex in answer to a deep inspiration was measured by laser Doppler fluximetry. The extent of vasoconstriction, measured at the second finger of the left hand, was expressed as percent reduction of the basal blood flux (% vasoconstriction). Insulin sensitivity was assessed before surgery in a subset of patients ( $n = 11$ ), by the method of euglycemic, hyperinsulinemic clamp. Body weight and composition were evaluated before, and 3, 6 and 12 months after surgery. At the same time points, energy intake (kJ/day) was evaluated by means of both food record diary and alimentary anamnesis.

**RESULTS:** The % vasoconstriction, which was significantly ( $P = 0.01$ ) greater in normoglycemic subjects than in diabetic ones, was also significantly ( $P = 0.03$ ) related to the extent of insulin sensitivity measured during the euglycemic clamp.

The % vasoconstriction showed a significant ( $P > 0.0001$ ), positive correlation with weight reduction obtained between the 6th and 12th months following surgery; as a consequence, % vasoconstriction was significantly ( $P = 0.0004$ ) related to the overall body weight loss achieved during the year following the operation. These correlations remained significant in multiple regression analysis on adjustment for age, initial body weight, plasma glucose and insulin ( $P = 0.0007$  and  $0.006$ , respectively). The % vasoconstriction was also significantly ( $P = 0.0006$ ), negatively related to energy intake measured 12 months after surgery.

**CONCLUSIONS:** In conditions of stable body weight, the sympathetic nervous system (SNS) reactivity is influenced by the degree of insulin resistance. A high capacity to activate the SNS, measured before surgery, is associated with both a larger gastric bypass-induced weight loss and a lower energy intake, at the phase of weight stabilization.

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## Introduction

The involvement of the sympathetic nervous system (SNS) in obesity has been investigated by several groups using various methods (see Snitker *et al*<sup>1</sup> and Van Baak<sup>2</sup> for review). For instance, an increase in energy intake elicits an increase in sympathetic activity, in normal body weight subjects.<sup>3,4</sup> This activation leads to enhanced energy expenditure, which

compensates the surplus energy intake and, therefore, prevents body weight gain. In obesity-prone subjects, a reduced SNS activity, as evaluated by the microneurography technique (MSNA), could contribute to the development of obesity.<sup>5</sup> Furthermore, in obese subjects, experimental over-feeding is not associated with an increase in sympathetic activity.<sup>6</sup>

A negative energy balance, due to a caloric restriction, is accompanied by a reduction of sympathetic activity at muscular level<sup>7</sup> and to an increased lipolysis in response to catecholamines in adipose tissue.<sup>8,9</sup> However, a reduced sensitivity to the lipolytic effect of catecholamines has been evidenced in young obese;<sup>10</sup> more importantly, the individual responsiveness to the catecholamine-induced lipolysis

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is predictive of the rate of body weight loss, during energy restriction.<sup>11</sup>

From all these data, it clearly appears that the SNS is sensitive to both positive and negative energy balance. Moreover, an impaired capacity of the SNS to adapt to modifications of energy balance could either contribute to the development of obesity and/or hamper body weight loss.

Therefore, we investigated the relationship between the sympathetic reactivity, measured before surgery by laser Doppler fluximetry (LDF), and the extent of body weight loss in a group of morbidly obese patients undergoing a surgical gastric bypass.

Recently, the evaluation of skin vasomotor reflexes by the LDF technique has been utilized to study the sympathetic component of the diabetic autonomic neuropathy.<sup>12</sup> In this study, the reduction of the capillary flux in response to a deep inspiration was assessed in a group of diabetic patients. This test evidenced that the vasoconstrictor reflex, which is mediated by a sympathetic activation, was markedly reduced in diabetic subjects, as compared to controls. The impaired vasoconstriction was significantly related to other tests of autonomic function, such as the postural fall in systolic blood pressure and the coefficient of variation of the R-R intervals at the heart level. This permitted the authors to propose the LDF as a sensitive and user-friendly method to study the peripheral sympathetic reactivity in diabetic subjects. On the other hand, it is well known that the variability of the R-R intervals is largely influenced by insulin resistance and obesity.<sup>13</sup> As a consequence, the already mentioned relationship between vasoconstrictor reflex and the variability of the R-R intervals suggested us that the vasoconstrictor reflex could also be affected by obesity and insulin resistance. We, therefore, utilized this technique to measure the sympathetic reactivity in a group of obese subjects before a Roux-en-Y gastric bypass.<sup>14</sup> We, then, investigated whether the capacity to activate the SNS could influence the subsequent surgically induced body weight loss.

## Subjects and methods

A total of 42 morbidly obese subjects (sex: 30 f/6 m; age: 39.2 ± 2y; body mass index (BMI) 46.01 ± 0.7 kg/m<sup>2</sup>) participated in the study. Among the group, 20 patients were hypertensive; eight of them were not treated and 12 received a non-beta blocker medication.

The experimental protocol received the approval of the local Ethical Committee and the volunteers gave their written consent.

Before surgery, skin blood flux modifications in response to a deep inspiration were measured at the level of the second finger of the left hand by LDF (MBF3D<sup>®</sup>, Moor, England). The experiments took place in a room maintained at a constant temperature of 22–24°C with the patient in sitting position. After a blood sampling and a 30-min rest period, basal blood flux, measured while the patient was

spontaneously breathing, was recorded and considered as 100% blood flux. The patient was then instructed to breath deeply and subsequently expire: this action induced a transient reduction of flux within few seconds; the minimal flux value was measured and expressed as % reduction of the basal flux (% vasoconstriction). Blood flux, then, spontaneously returned to the basal level. Deep inspiration was repeated six times, at intervals of 1 min, in each patient. The % vasoconstriction was the mean of the six measurements.

Plasma glucose was determined enzymatically,<sup>15</sup> and insulin by RIA.<sup>16</sup> In a smaller group of patients ( $n=11$ ), insulin sensitivity was quantified during a euglycemic, hyperinsulinemic clamp<sup>17</sup> as the amount of glucose (glucose uptake) infused in order to maintain a constant glycemia of 5 mM in the presence of elevated insulin levels within the physiological range. Glucose uptake was expressed as a function of the lean body mass (ie mg/min/kg of LBM). To do so, lean body mass (kg) was evaluated by bioimpedance according to Segal *et al.*<sup>18</sup>

Body weight was measured before, and 3, 6 and 12 months after the surgical intervention. Body weight loss was expressed either in absolute values (delta bw, kg) or as % correction of excess body weight. Excess body weight was calculated as the difference between measured body weight and normal body weight, where normal body weight was considered to correspond to a BMI of 25 kg/m<sup>2</sup>.

Food consumption was evaluated before, and 3, 6 and 12 months after surgery as follows: patients were instructed to record their food consumption during three consecutive days; then, they were interviewed by a dietitian in order to obtain a detailed diet recall. Data so obtained were analyzed by a computer program (Prodi 3) to calculate the daily caloric intake (En.In., MJ/day).

Results are expressed as mean ± s.e.m.; factorial ANOVA and simple or multiple regression analysis were utilized for the analysis of the results.

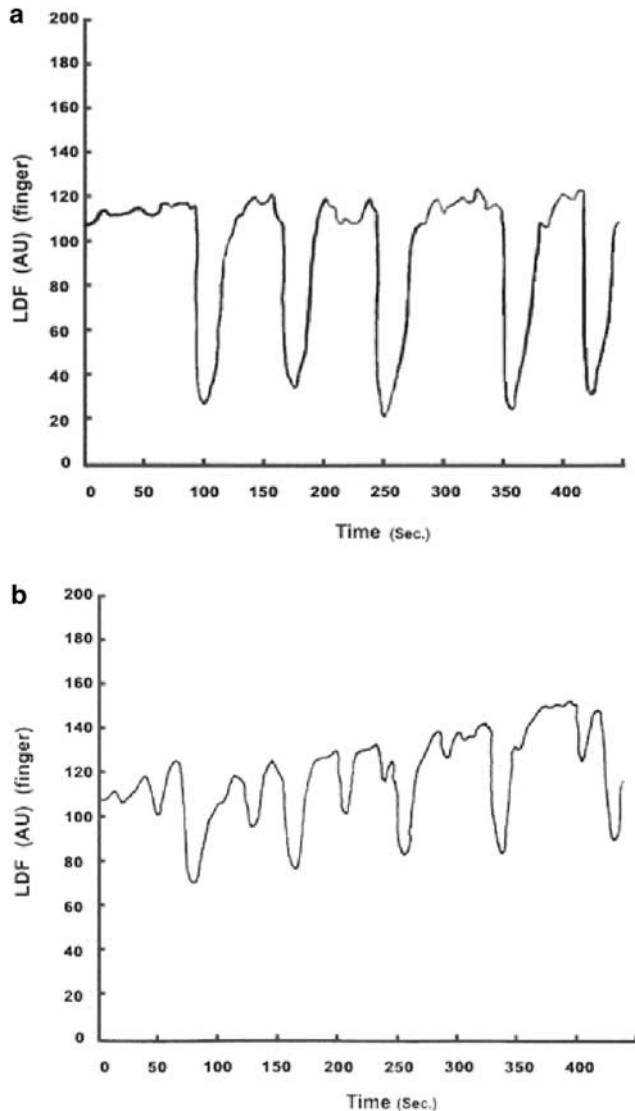
## Results

In basal, preoperative conditions, patients were divided into three groups, according to their fasting glycemia (Table 1).<sup>19</sup> An example of the tracings recorded in normoglycemic (a) or in diabetic (b) subjects is displayed in Figure 1. Factorial ANOVA showed that, in diabetics (ie fasting glycemia > 7.0 mM), LDF-measured % vasoconstriction was significantly ( $P=0.002$ ) reduced, when compared to the normoglycemic group (ie fasting glycemia < 6.0 mM); no significant difference was observed in the group with impaired fasting glycemia (ie fasting glycemia between 6 and 7 mM). This result indicated a relationship between insulin sensitivity and the capacity to activate the SNS. This was confirmed by the results obtained in a smaller group of obese female patients ( $n=11$ , age: 35 ± 3y, bw: 119.0 ± 3.0 kg) in whom insulin sensitivity was measured by the euglycemic, hyperinsulinemic clamp technique. In these patients (Figure 2), the % vasoconstriction was

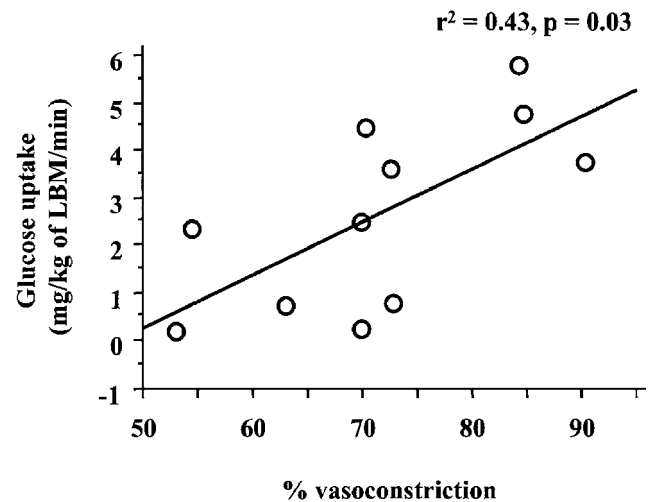
**Table 1** Physical and metabolic characteristics and sympathetic reactivity (% vasoconstriction as assessed by LDF) measured before surgery in the study group divided into three subgroups according to fasting glycaemia

	Normal fasting glycaemia (n = 27)	Impaired fasting glycaemia (n = 9)	Diabetes (n = 6)
Age (y)	37.5 ± 1.9	39.3 ± 3.7	47.0 ± 3.6*
Body weight (kg)	121.5 ± 3.7	128.0 ± 4.9	129.7 ± 5.2
LDF-measured			
% vasoconstriction	79.6 ± 2.1	74.2 ± 3.3	63.5 ± 3.9*
Plasma glucose (mM)	4.8 ± 0.1	6.2 ± 0.1*\$\$	11.2 ± 1.4***
Plasma insulin (ng/ml)	1.6 ± 0.2	1.6 ± 0.2\$	2.8 ± 0.6*

\*: significantly different from normal fasting glycaemia group; \$: significantly different from diabetes group; one symbol  $P < 0.05$  or  $P < 0.01$ , two symbols  $P < 0.0001$ .

**Figure 1** Typical example of LDF tracings recorded in (a) normoglycemic and (b) diabetic subjects.

significantly ( $r^2 = 0.43$ ,  $P = 0.03$ ) related to the glucose uptake, measured during the steady-state period of the euglycemic clamp.

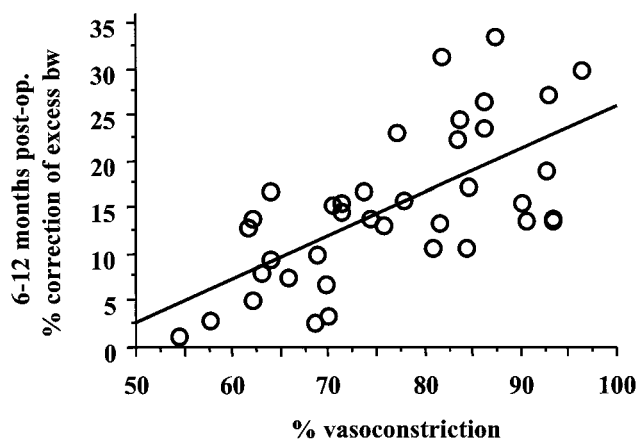
**Figure 2** Relationship between sympathetic reactivity (% vasoconstriction), measured by LDF and insulin sensitivity (glucose uptake mg/kg of LBM/min), measured by euglycemic, hyperinsulinemic clamp. Both measurements were performed before surgical intervention of gastric bypass.**Table 2** Evolution of body weight during 1 y after gastric bypass

	Basal	3 months	6 months	12 months
Body weight (kg)	124.1 ± 2.7	103.7 ± 2.5	95.0 ± 2.4	86.5 ± 2.5
% correction of excess bw	—	38.2 ± 1.3	53.8 ± 1.8	69.1 ± 2.6

As shown in Table 2, surgical intervention induced a marked decrease in body weight during the subsequent 12-month period. At 1 y after surgery, mean weight loss was of  $39.3 \pm 1.5$  kg, which corresponded to a  $69.2 \pm 2.6\%$  correction of the initial body weight excess.

Univariate regression analysis evidenced a significant ( $r^2 = 0.44$ ,  $P < 0.0001$ ) relationship between the % vasoconstriction, measured before surgery, and the correction of excess body weight obtained during the 6–12-month postoperative period (Figure 3). As a consequence, the sympathetic reactivity showed a significant ( $r^2 = 0.25$ ,  $P = 0.001$ ) impact on the extent of reduction of overweight

$r^2 = 0.44, p < 0.0001$



**Figure 3** Relationship between sympathetic reactivity (% vasoconstriction), measured by LDF before surgery and % correction of excess body weight obtained during the 6–12-month period following gastric bypass.

**Table 3** Multiple regression analysis of body weight loss during the 6–12-month postoperative period vs various parameters measured in preoperative conditions

Dependent variable	Independent variables	Coefficient	P-value
6–12-month % correction exc. bw	LDF-measured % vasoconstriction	0.43	0.0006
	Initial body weight (kg)	−0.05	0.46
	Age (y)	−0.06	0.66
	Glycemia (mM)	0.64	0.25
	Insulinemia (ng/ml)	−2.08	0.18
	Mean blood pressure (mmHg)	−0.08	0.39

achieved during the overall 12-month postoperative period. This in spite of the lack of significant relationship between pre-operative sympathetic reactivity and weight reduction obtained during the first 6 months after surgery ( $P = 0.15$ , NS). The significant relationship between % vasoconstriction and weight reduction persisted when the latter was expressed in absolute (kg) values:  $r^2 = 0.33$ ,  $P = 0.0002$  and  $r^2 = 0.10$ ,  $P < 0.05$ , respectively, for the 6–12-month period and for the overall 12-month period.

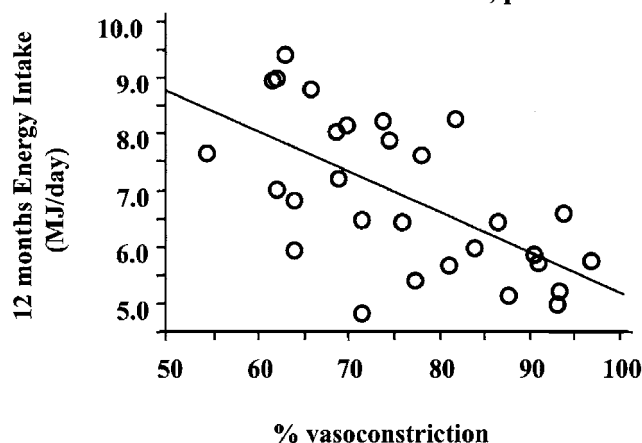
Multiple regression analysis demonstrated that % vasoconstriction remained independently and significantly related to both the 6–12-month ( $P = 0.0006$ , Table 3) and the 1-y ( $P = 0.02$ , Table 4) % correction of excess body weight, once that age, initial body weight and preoperative values of glycemia, insulinemia and blood pressure were taken into account.

In univariate regression analysis, only initial body weight showed a significant ( $P = 0.01$ ), negative relationship with % correction of excess body weight at 12 months. No

**Table 4** Multiple regression analysis of body weight loss during the overall 12-month postoperative period vs various parameters measured in preoperative conditions

Dependent variable	Independent variables	Coefficient	P-value
Overall 12-month % correction exc. bw	% vasoconstriction	0.57	0.02
	Initial body weight (kg)	−0.31	0.04
	Age (y)	−0.27	0.32
	Glycemia (mM)	0.77	0.50
	Insulinemia (ng/ml)	−0.04	0.98
	Mean blood pressure (mmHg)	0.10	0.60

$r^2 = 0.39, p = 0.0003$



**Figure 4** Relationship between sympathetic reactivity (% vasoconstriction), measured by LDF before surgery and energy intake (MJ/day) measured 12 months after surgery.

significant relationships were observed with glycemia, insulinemia and age.

Finally, as shown in Figure 4, % vasoconstriction showed a significant ( $r^2 = 0.39$ ,  $P = 0.0003$ ) relationship to the energy intake measured at 12 months after surgery, whereas no significant relationships were observed with energy intake measured before and 3 and 6 months after surgery.

When patients were divided into two categories, according to their blood pressure, hypertensive group showed a significant reduction of the vasoconstrictive reflex ( $79.8 \pm 2.4$  vs  $72.1 \pm 2.5\%$ , respectively, in normotensive and hypertensive groups,  $P = 0.03$ ). However, when only normoglycemic subjects were compared, no significant differences were observed in their vasoconstrictive response to deep breath (normoglycemic, normotensive: % vasoconstriction =  $79.9 \pm 2.9\%$  ( $n = 20$ ) vs normoglycemic, hypertensive: % vasoconstriction =  $78.9 \pm 3.6\%$  ( $n = 7$ ), NS). This indicates that the difference observed between hypertensive and normotensive subjects when examining the whole study group is linked to the presence, in the hypertensive group, of the diabetic subjects.

## Discussion

Our results clearly show the great variability of the SNS reactivity in morbidly obese patients. This strongly suggests that, besides obesity, other factors exert an influence on the sympathetic function. In particular, our results show that an impaired insulin sensitivity is associated with a progressive reduction in the capacity to activate the SNS in obese subject. A previous study<sup>20</sup> has shown an association between reduced insulin sensitivity and reduced responsiveness of the parasympathetic nervous system. In fact, it has been demonstrated that the acetylcholine-mediated vasodilation, measured by LDF, is significantly, positively related to insulin sensitivity, as measured by euglycemic hyperinsulinemic clamp. By using the same techniques, we could demonstrate that the reactivity of the sympathetic branch of the autonomic nervous system, measured as % vasoconstriction, is also affected by insulin resistance.

Even though the measurement of deep inspiration-induced vasoconstriction by LDF provides a reliable evaluation of the responsiveness of the SNS, this technique does not give information about the basal, spontaneous nerve activity. We are therefore unable to establish whether the decreased sympathetic reactivity observed in obese diabetic patients is associated or not with an elevated basal sympathetic tonus. However, several papers have demonstrated that the increased sympathetic tonus, well documented in obese subjects, is often associated with a decreased capacity to further enhance sympathetic activity in answer to different stimuli, such as insulin<sup>13,21,22</sup> or phenylephrine.<sup>23</sup>

Nevertheless, our results show that a good capacity to activate the SNS, measured in condition of stable body weight, is associated with a larger weight loss after gastric bypass. To our knowledge, this is the first report clearly demonstrating the determinant role played by the SNS in the weight loss process induced by bariatric surgery. Astrup *et al*<sup>24</sup> found that circulating catecholamines are positively associated with hypocaloric diet-induced body weight loss, but this relationship was no longer significant when other factors such as energy expenditure or plasma dihydrotestosterone were taken into account. In our study, multiple regression analysis confirmed the significant impact of sympathetic reactivity on weight loss outcome of surgically treated obesity, even when initial body weight, plasma insulin and glucose values are taken into account.

Blood pressure could also affect sympathetic reactivity, as suggested by the significant ( $P=0.03$ ) difference between hypertensive and normotensive group subjects. This difference, however, seems to be more linked to the presence of diabetic subjects in the hypertensive group, rather than to an effect of blood pressure *per se*: in fact, when only normoglycemic subjects are investigated, no significant differences between hypertensive and normotensive are observed.

The mechanism by which the SNS exerts its positive influence on weight reduction remains to be elucidated. It has been demonstrated that fasting increases norepinephrine

spillover selectively from the adipose tissue;<sup>25</sup> furthermore, the beta-adrenergic-stimulated lipolysis is enhanced during hypocaloric diet;<sup>9</sup> these phenomena could be particularly evident in patients with a high degree of sympathetic reactivity.

Hellström *et al*<sup>11</sup> submitted a group of obese patients to a very low-calorie diet and demonstrated that the subjects who achieved the largest weight reduction were those who had a decreased alpha-2 adrenoreceptor sensitivity to catecholamine stimulation at the adipose tissue level before the diet. The authors suggest that an imbalance between the alpha and beta adrenoreceptors of the adipose tissue could lead to a prevalent beta-adrenergic-mediated lipolytic effect and, therefore, favor a larger body weight loss. Further investigations are needed in order to establish whether one or the other of the mechanisms proposed by these studies was operating in the patients who, in our study, showed both the highest sympathetic excitability and the largest weight reduction.

During severe caloric restriction, energy expenditure is reduced<sup>26</sup> and this energy economy leads to a lower extent of weight loss;<sup>27</sup> a high degree of sympathetic reactivity could prevent energy economy and, therefore, allow a larger weight reduction.

It is known that meal ingestion increases sympathetic activity;<sup>28</sup> in turn, sympathetic beta activation induces satiety,<sup>29</sup> at least in rats. This suggests that SNS is involved in the control of meal size. In the present study, sympathetic reactivity was significantly related to energy intake, measured 12 months following surgery. This result is potentially of interest, when considering that, in the majority of the patients, 1y after surgery the body weight loss process is achieved and the phase of weight stabilization begins. In fact, the persistence of a low-caloric intake, associated with a high sympathetic reactivity, could lead to a greater body weight loss by extending the period of negative energy balance. In addition, it is clear that the final body weight and/or a possible weight relapse are greatly influenced by the energy intake at the stabilization period. The reduction of energy intake is the main effect of this surgical intervention:<sup>30</sup> in a previous study,<sup>31</sup> we demonstrated that post-operative food intake shows a great individual variability and is influenced by age; in the present study, we suggest that sympathetic excitability could also play a role in the control of energy intake during the stabilization period.

In conclusion, SNS reactivity promotes a greater body weight loss following gastric bypass. Together with our previous results, the present study underlines that besides a highly standardized surgical intervention, the weight loss of surgically treated obesity is influenced by several characteristics such as age and initial body weight,<sup>31</sup> their capacity to spare energy<sup>27</sup> and to activate the sympathetic nervous system. These findings could be useful to better understand the large individual variability of body weight loss obtained by bariatric surgery and, more generally, by any weight reduction program.

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