High-fat diet-induced obesity leads to increased NO sensitivity of rat coronary arterioles: role of soluble guanylate cyclase activation

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1Second Department of Medicine and Center of Cardiology, University of Szeged, Szeged; 2Institute of Cardiology, University of Debrecen, Debrecen; 3Department of Pathophysiology and Gerontology, University of Pecs, Pecs, Hungary; and 4Department of Physiology, New York Medical College, Valhalla, New York

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Jebelovszki E, Kiraly C, Erdei N, Feher A, Pasztor ET, Rutkai I, Forster T, Edes I, Koller A, Bagi Z. High-fat diet-induced obesity leads to increased NO sensitivity of rat coronary arterioles: role of soluble guanylate cyclase activation. Am J Physiol Heart Circ Physiol 294: H2558–H2564, 2008. First published April 11, 2008; doi:10.1152/ajpheart.01198.2007.—The impact of obesity on nitric oxide (NO)-mediated coronary microvascular responses is poorly understood. Thus NO-mediated vasomotor responses were investigated in pressurized coronary arterioles (~100 μm) isolated from lean (on normal diet) and obese (fed with 60% of saturated fat) rats. We found that dilations to acetylcholine (ACH) were not significantly different in obese and lean rats (lean, 83 ± 4%; and obese, 85 ± 3% at 1 μM), yet the inhibition of NO synthesis with Nω-nitro-l-arginine methyl ester reduced ACH-induced dilations only in vessels of lean controls. The presence of the soluble guanylate cyclase (sGC) inhibitor oxadiazolo-quinoxaline (ODQ) elicited a similar reduction in ACH-induced dilations in the two groups of vessels (lean, 60 ± 11%; and obese, 57 ± 3%). Dilations to NO donors, sodium nitroprusside (SNP), and diethylenetriamine (DETA)-NONOate were enhanced in coronary arterioles of obese compared with lean control rats (lean, 63 ± 6% and 51 ± 5%; and obese, 78 ± 5% and 70 ± 5%, respectively, at 1 μM), whereas dilations to 8-bromo-cGMP were not different in the two groups. In the presence of ODQ, both SNP and DETA-NONOate-induced dilations were reduced to a similar level in lean and obese rats. Moreover, SNP-stimulated cGMP immunoreactivity in coronary arterioles and also cGMP levels in carotid arteries were enhanced in obese rats, whereas the protein expression of endothelial NOS and the sGC β2-subunit were not different in the two groups. Collectively, these findings suggest that in coronary arterioles of obese rats, the increased activity of sGC leads to an enhanced sensitivity to NO, which may contribute to the maintenance of NO-mediated dilations and coronary perfusion in obesity.

microcirculation; nitric oxide; guanosine 3′,5′-cyclic monophosphate; dilatation

There is a general agreement that obesity confers increased risk for developing cardiovascular disease and its complications, such as coronary heart disease (17). Vasomotor dysfunction of the coronary microvessels is considered to be one of the early developing alterations in obesity, contributing to the disturbed regulation of tissue perfusion and predisposing patients to myocardial ischemia (9). Studies have shown that any increase in body mass requires higher cardiac output and consequently increased coronary blood flow (14, 20, 22). Given that, an impairment of coronary vasomotor function is likely to be more detrimental on myocardial perfusion in obese subjects. In contrast, it has been reported that cardiovascular function in obese and hypertensive patients may not be impaired compared with that in lean and hypertensive individuals, and it has been proposed that obesity, in some cases, may protect patients from the deleterious vascular effect of hypertension by decreasing hypertensive target organ damage (4, 22). Thus it is likely that a functional adaptation of the vascular system develops in obesity, which, at least in the early phase, provides an adequate tissue perfusion to meet the higher metabolic requirements (22).

The existence of such vascular adaptive mechanisms in coronary vessels was substantiated by our recent observations showing an enhanced dilator capacity of coronary microvessels isolated from diabetic patients (31) and also in obese patients with hypertension (15). Particularly, we have found that coronary arteriolar dilations to bradykinin and also to the nitric oxide (NO) donor sodium nitroprusside (SNP) were augmented in obese patients and were positively correlated with the body mass index (15). In line with our observations, there are few recent reports showing not only preserved (18, 19, 21, 34) but even enhanced (26) coronary vasodilations in different animal models of obesity, although the underlying mechanisms remain obscure.

These aforementioned findings led us to the hypothesis that obesity activates, as yet unknown, adaptive mechanisms intrinsic to the coronary arteriolar wall, aiming to maintain adequate tissue perfusion of the myocardium. Accordingly, in this study, we set out to characterize the impact of obesity on coronary arteriolar vasomotor function, hence to furnish evidence for vascular adaptation and to explore the possible cellular mechanisms involved. Since NO plays an important role in regulating coronary blood flow, we have focused the investigation on the possible alterations in NO-mediated vasomotor function in isolated, pressurized coronary arterioles of lean and high-fat diet-induced obese rats (10).

METHODS

Animal model of obesity. Male Wistar rats (n = 50) were purchased from Charles River. The rats were maintained in the animal care facility at our university with a 12-h:12-h light-dark cycle and were given free access to food and water. The rats were maintained on standard rat chow (n = 25) or on high-fat diet (n = 25; European Union-modified rodent diet with 60% fat; 58Y1, TestDiet; PMI Nutrition) for 10 wk (10). All protocols were approved by the Institutional Animal Care and Use Committee.

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**Isolation of rat coronary arterioles.** With the use of microsurgical instruments and an operating microscope, the second branch of the septal artery (~1.5 mm in length) running intramuscularly was isolated and cannulated. The cannulated arteriole was connected with silicone tubing to a pressure servo control system (Living Systems Instrumentation) to set the intraluminal pressure to 80 mmHg. Changes in arteriolar diameter were continuously recorded with a digital camera (CFW1310; Scion) connected to a microscope (Eclipse 80i; Nikon) (2).

**Assessment of coronary arteriolar responses.** During an incubation period of 1 h, a spontaneous myogenic tone developed in the isolated coronary arterioles in response to the intraluminal pressure of 80 mmHg. Cumulative concentrations of the endothelium-dependent vasodilator acetylcholine (ACh; 1 nmol/l–1 μmol/l) were administered to the coronary arterioles from lean and obese rats in the presence and absence of Nω-nitro-L-arginine methyl ester (l-NAME; 200 μmol/l for 30 min), an inhibitor of the NO synthase, and changes in diameter were measured. Arterioles were then incubated with the soluble guanylate cyclase (sGC) inhibitor oxadiazolo-quinoxaline (ODQ; 10 μmol/l–30 min), and arteriolar responses to ACh were obtained again in the two groups. In a separate set of experiments, dilutions to cumulative concentrations of NO donors SNP (1 nmol/l–10 μmol/l) and DETA-NONOate (1 nmol/l–10 μmol/l) were investigated in isolated coronary arterioles of lean and obese rats. The arterioles were then incubated with ODQ (10 μmol/l for 30 min), and arteriolar responses to NO donors were reassessed. In another series of experiments, increasing concentrations of 8-bromo-cGMP, a cell-permeable cGMP analog (1 nmol/l–10 μmol/l), were administered and changes in diameter were measured.

**cGMP immunocytochemistry.** The sGC activity was detected in coronary arterioles through the identification of basal and NO donor-stimulated increases in cGMP immunoactivity by using antibody against cGMP similarly as it was described previously (12). Briefly, the left ventricle including the coronary arteriole was embedded and frozen in optimal cutting temperature compound (Tissue Tek; Electron Microscopy Sciences). Unfixed consecutive sections (10 μm thick) were transferred to a solution containing 1 μM SNP in PBS and incubated for 15 min at 37°C. Other sections remained in PBS solution during the 15-min incubation period and served as unstimulated controls. Sections were then fixed with acetone and immunolabeled with a monoclonal anti-cGMP primary antibody (dilution 1:2,000; Sigma). Immunostainings were visualized by using an avidin-biotin horseradish peroxidase visualization system (Vectorstain kit; Vector) and stained with diaminobenzidine (DAB). For nonspecific binding, the primary antibody was omitted. Images of the sections were collected with a digital camera (CFW 1310C; Scion) connected to a Nikon Eclipse 80i microscope. For a semiquantitative analysis of the cGMP immunoreactivity, in defined areas of the arteriolar wall (6 separate regions in each arteriole with diameter of ~100–150 μm), the amount of the brown product (DAB) was estimated by measuring optical density using the National Institutes of Health (NIH) Image software. The background (absence of the first antibody) was subtracted, and the averaged optical density was then calculated and compared in coronary arterioles of lean and obese rats.

**cGMP ELISA.** The sGC activity was detected in the carotid artery through the identification of basal and NO donor, SNP-stimulated increases in cGMP levels, which was measured with a commercially available ELISA kit (Assay Design) following the instructions by the manufacturer.

**Immunoblots.** Single coronary arteries (1 vessel from each animal) were dissected from the hearts of lean and obese rats, cleared of connective tissue, and briefly rinsed in ice-cold physiological salt solution. After the addition of 20 μl of Laemmli sample buffer (Sigma), the tissues were homogenized. Immunoblot analysis was carried out as described before (3). The antibodies used for the detection of protein expression [anti-endothelial NOS (eNOS) IgG and anti-sGC β1-subunit IgG] were obtained from Sigma. Anti-β-actin IgG obtained from Abcam was used as a loading control. Signals were revealed with chemiluminescence and visualized autoradiographically. The optical density of the bands was quantified and normalized for β-actin by using NIH Image software.

**Statistical analysis.** Statistical analyses were performed using GraphPad Prism Software (San Diego, CA) by two-way repeated-measures ANOVA, followed by Tukey’s post hoc test or Student’s t-test as appropriate. Data are expressed as means ± SE. In isolated vessels, agonist-induced arteriolar responses were expressed as changes in arteriolar diameter as a percentage of the maximal dilatation defined as the passive diameter of the vessel at 80 mmHg intraluminal pressure in a Ca2+-free medium. P < 0.05 was considered statistically significant.

**RESULTS**

After a commencement of the high-fat diet for 10 wk, the body weight, serum insulin, glucose, and total cholesterol levels of rats became significantly greater compared with those of rats fed the standard diet (Table 1), similar to the findings observed in our previous study (10). We have also found that the C-reactive protein (CRP) levels were similar in the two groups of animals (Table 1). It should be noted that we have found a significant elevation in fasting glucose levels in this model of diet-induced obesity. However, the glucose levels were only slightly elevated in obese rats (Table 1) compared with other animal models of Type 2 diabetes, such as the db/db mice (2) or the diabetic Zucker rats (13), in which animals have about four times higher fasting glucose levels.

**Coronary arteriolar responses to ACh.** In coronary arterioles isolated from lean and obese rats, there were no significant differences between the spontaneously developed arteriolar tone (86 ± 6 and 97 ± 6 μm, respectively) and in the passive arteriolar diameters (in Ca2+-free medium, 153 ± 9 and 149 ± 7 μm at 80 mmHg intraluminal pressure, respectively). We have found that endothelium-dependent dilations to ACh were not significantly different between the coronary arterioles of lean and obese rats (Fig. 1A). The inhibition of NO synthesis with l-NAME decreased ACh-induced dilation in coronary arterioles isolated from lean animals (Fig. 1B), whereas it had no significant effect on ACh-induced responses in arterioles of obese rats (Fig. 1C). The administration of ODQ, an inhibitor of sGC, elicited a similar reduction in ACh-induced dilations in the coronary arterioles of the two groups of animals (Fig. 1D).

**Coronary arteriolar responses to NO donors.** Dilations to the NO donors, SNP, and DETA-NONOate were also tested and interestingly found to be significantly increased in the arterioles isolated from obese compared with lean rats (Fig. 2, A and B). SNP- and DETA-NONOate-induced dilations were also obtained after the administration of ODQ, an inhibitor of sGC.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Lean</th>
<th>Obese</th>
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<tbody>
<tr>
<td>Body weight, g</td>
<td>376±16</td>
<td>569±17*</td>
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<tr>
<td>Serum glucose, mmol/l</td>
<td>6.5±0.3</td>
<td>9.6±0.7*</td>
</tr>
<tr>
<td>Serum insulin, pmol/l</td>
<td>91±11</td>
<td>221±19*</td>
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<tr>
<td>Serum total cholesterol, mmol/l</td>
<td>1.20±0.08</td>
<td>1.62±0.07*</td>
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<td>C-reactive protein, mg/l</td>
<td>0.12±0.01</td>
<td>0.14±0.01</td>
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Values are means ± SE; n = 20 lean (normal) and 20 obese (high-fat diet) rats per group. *P < 0.05, significant difference.
ODQ decreased SNP- and DETA-NONOate-induced dilations in both groups to the same level, thereby eliminating the NO donor-evoked differences in dilations between the two groups (Fig. 2, A and B). In a separate series of experiments, arteriolar responses were obtained to 8-bromo-cGMP, a cell-permeable, stable cGMP analog. We found that 8-bromo-cGMP elicited substantial dilations in coronary arterioles, which were not, however, significantly different in the two groups (Fig. 2 C).

Immonocytochemistry. Basal and SNP-stimulated cGMP immunoreactivity were detected in a native coronary arteriolar section in lean and obese rats. No specific labeling was detected in the section in which the first antibody was omitted. We have found that SNP-stimulated cGMP immunoreactivity was increased in the coronary arterioles of lean and obese rats, and the enhancement was found to be greater in the coronary arterioles of obese rats (Fig. 3, A and B).

cGMP ELISA. Basal and SNP-stimulated cGMP levels were directly measured in the carotid artery of lean and obese rats. We have found that basal cGMP levels were similar in vessels from lean and obese rats (Fig. 3C). The NO donor SNP elicited marked increases in cGMP levels in both groups, which tended to be increased in those of vessels from obese rats (Fig. 3C).

Western immunoblots. Western blot analysis was performed in single coronary arteries from both lean and obese rats. We have found that there were no significant differences in the eNOS protein expression (Fig. 4A) or in the sGC β1-subunit protein levels in the coronary arterioles of lean and obese rats (Fig. 4B).

DISCUSSION

In this study, we set out to characterize the impact of high-fat diet-induced obesity on NO-mediated coronary arteriolar dilations, known to contribute substantially to the regulation of coronary blood flow (33). We have found that when we compared responses of lean and obese rats, coronary arteriolar dilations to the endothelium-dependent dilator ACh in the obese rat were essentially preserved but not significantly affected by NO synthase inhibition, whereas the dilations to NO donors, SNP, and DETA-NONOate were significantly enhanced and reduced by the sGC inhibitor ODQ to the control level. In addition, we have found that NO donor-stimulated vascular cGMP immunoreactivity and cGMP levels were increased in obese rats. Collectively, these findings indicate that in high-fat diet-induced obesity, due to the increased sGC activity, the NO sensitivity of coronary arterioles is enhanced. Thus, despite the impaired NO bioavailability, this adaptive mechanism may contribute to the preserved ACh-induced vasodilation and also to the maintenance of NO-mediated vascular signaling.

It seems well established that obesity and Type 2 diabetes are associated with the impaired bioavailability of NO both in conduit vessels and resistance arteries of the periphery (2, 18, 27–29, 32). In this context, previous studies have demonstrated that impaired NO availability ultimately leads to reduced...
agonist-induced dilations of cerebral, mesenterial (11, 24), and skeletal muscle microvessels (13). Recently, we have reported that in high-fat diet-induced obese rats, skeletal muscle arterioles also exhibit reduced endothelium-dependent agonist (ACh and histamine)-induced, NO-mediated dilations (10).

In contrast, studies obtained on coronary vessels in various pathological conditions are seemingly inconsistent in the literature. Impaired coronary dilations have been found in animal models of diabetes and prediabetes (16, 25, 37). In contrast, our recent observations show an enhanced dilator capacity of coronary microvessels isolated from diabetic patients (31) and also in obese patients with hypertension (15). Similarly, there are reports showing not only preserved (18, 21) but even enhanced (26) coronary vasodilations in animal models of obesity and diabetes mellitus. The possible mechanisms explaining these aforementioned discrepancies, however, remained obscure and may be explained by the various models (i.e., animals vs. humans) or the different disease states used (obesity, prediabetes, and manifest Type 2 diabetes) in those of the aforementioned experiments.

In this study, we have found that in coronary arterioles (active diameter, <100 μm) obtained from high-fat diet-treated obese rats, ACh-induced dilation was essentially preserved (Fig. 1A). No significant changes in the protein expression of eNOS were also detected by Western blot analysis in these coronary microvessels (Fig. 4A). Interestingly, we have found that in the coronary arterioles of the obese rats, the pharmacological inhibition of NOS had no significant effect of ACh-induced dilations (Fig. 1C), whereas it reduced those...
responses in control vessels (Fig. 1B). This finding suggested either the lack of NO mediation or a reduction of the amount of the NO production in obese animals, which cannot, however, be detected by measuring diameter changes of arterioles in the presence of a NOS inhibitor. On the other hand, this finding also raised the possibility that in coronary arterioles of obese rats, unlike those of arteriolar responses in the skeletal muscle, mechanisms intrinsic to the vascular wall are activated to compensate for the reduced NO availability. In the coronary circulation, oxygen extraction is near maximal (33), and an impairment of arteriolar dilator function could have a significant consequence on tissue perfusion, leading to ischemia. It is also known that any increase in body mass (muscular or adipose tissue) requires a higher cardiac output and expanded intravascular volume to meet the elevated metabolic requirements (22). Given that, in obesity, coronary resistance vessels should adopt to increased coronary blood flow and metabolic requirements to maintain adequate tissue perfusion during the disease development. Our recent study provided evidence for the existence of an adaptation of human coronary microvessels isolated from obese patients by showing enhanced dilations to the NO donor SNP compared with those of lean individuals (15). Collectively, our previous and present findings suggest that in obesity, both in humans and animal models, adaptive mechanisms are activated in the wall of coronary microvessels to maintain or enhance their dilator capacity.

It should be noted that the overall vasodilator capacity of coronary circulation has not been determined in this study, which can be only evaluated in vivo. It is known that alterations in the morphology of coronary microvessels, such as changes in the lumen cross-sectional area or vascular rarefaction, all may have an impact on the overall blood flow capacity in the coronary circulation (6). On the basis of our present findings obtained in isolated vessels, we can only speculate whether the overall coronary dilator capacity is preserved or even enhanced in vivo, an idea that has yet to be tested in future studies of obesity.

In the present study, an attempt was also made to elucidate the possible underlying mechanism contributing to the observed functional adaptation of coronary microvessels in obese rats. Our findings show that in obese rats, the sensitivity of coronary arterioles to NO was significantly enhanced, as demonstrated by augmented vasodilations to NO donors, SNP, and DETA-NONOate (Fig. 2). Interestingly, in obese patients, we have obtained similar results, namely that NO donor-induced coronary arteriolar and also brachial artery dilations were significantly enhanced (15). Enhanced dilations of coronary arterioles to the NO donor SNP have also been described in female pigs fed with high-fat diet (36). Collectively, these data suggest that an impaired NO availability in coronary microvessels of obese subjects can be associated with an enhanced NO sensitivity of the coronary arterioles, and this mechanism may responsible for the maintained agonist-induced dilations, also found in the present study. Increased sensitivity for NO has been already proposed in previous observations in different conditions associated with impaired NO availability. For instance, Brandes et al. (7) reported that both the acute cessation of endothelial NO production in wild-type mice and the chronic deficiency of NO in eNOS knockout mice increase the NO sensitivity of vascular smooth muscle cells in response to nitrovasodilator agents. They concluded that an enhanced sensitivity of the downstream sGC to NO may compensate for the reduced NO availability (7). In this context, it has also been found that the acute administration of exogenous NO decreased sGC activity and, long term, its protein expression (30). These findings indicate that NO may play an important, negative feedback regulatory role on the catalytic activity of its effector sGC; hence, any reduction of the NO level may lead to an enhancement of the sensitivity of sGC to NO.

To demonstrate the possible involvement of enhanced sGC activation, our present study revealed that the inhibition of sGC by ODQ reduced both ACh- and also NO donor (SNP and NONOate)-induced arteriolar dilations and, importantly, eliminated differences between the responses of coronary arterioles of lean and obese rats (Figs. 1C and 2B). We have also found that the stable cGMP analog 8-bromo-cGMP elicited substantial dilations in coronary arterioles, which were not, however, significantly different in the two groups of vessels (Fig. 2C). These findings indicate the potential involvement of enhanced sGC activation in mediating the enhanced sensitivity of smooth muscle cells for NO in coronary arterioles of obese rats. On the basis of the present findings, only a minor, if any, role can be ascribed for the contribution of other mechanisms, such as the peroxynitrite-dependent, S-glutathiolation-mediated activation of the sarco(endo)plasmic reticulum Ca2+−ATPase, which may also lead to an enhanced NO sensitivity, independent of sGC activation, as suggested previously (1). Furthermore, our cytochemistry data suggested a maintained or even enhanced SNP-stimulated cGMP immunoreactivity in the coronary arterioles of obese rats (Fig. 3, A and B). To quantitate this observation in parallel experiments, basal and stimulated cGMP contents were also measured in carotid arteries of lean and obese rats with cGMP ELISA. In this assay, we have found no significant differences in the basal cGMP content but detected elevated cGMP levels in SNP-stimulated vessels of lean and obese rats (Fig. 3C). Although the results obtained in a conduit vessel cannot be directly extrapolated to those of coronary microvessels, these data are in accordance with immunocytochemistry data obtained in coronary arterioles and suggest maintained or even increased sGC activation in obese animals. The results showing no changes in the protein expression of the sGC β1-subunit (Fig. 4B), along with the findings that 8-bromo-cGMP-evoked coronary dilations were similar in lean and obese rats (Fig. 2C), suggest a primary role for an enhanced activity of sGC enzyme in the coronary arterioles of obese rats.

An intriguing question, namely, what are the exact origin and nature of factor(s) that may contribute to the activation of sGC, is still open. It seems plausible that the lack of NO may lead to enhanced sGC sensitivity (7). It has been shown that TNF-α-mediated vascular inflammation has important consequences with respect to the downstream signaling of NO (25). In this study, an attempt was made to estimate the level of systemic inflammation likely to be present in obese rats. To this end, we have measured the serum levels of CRP, which was found, however, to be comparable in lean and obese animals (Table 1). This data suggest that in this model, a manifest systemic inflammation is unlikely present, although a possible involvement of inflammation localized in the vascular wall cannot be entirely excluded and has yet to be elucidated in future studies.

Moreover, it has been proposed that oxidative and/or nitrosative stress may lead to the inactivation of both sGC and AJP-Heart Circ Physiol • VOL 294 • JUNE 2008 • www.ajpheart.org
cGMP-dependent protein kinase I. On the other hand, it has been demonstrated that acute exposure of hydrogen peroxide can lead to the direct activation of sGC, contributing to the relaxation of healthy bovine pulmonary arteries (8, 35). Moreover, Baurersachs et al. (5) have shown that myocardial infarct-induced ischemic heart failure in rats leads to the upregulation of both vascular eNOS and sGC expression in association with an increased vascular superoxide production. Our previous study showed that high-fat diet-treated rats exhibit an enhanced xanthine oxidase-derived superoxide anion production (10). Thus it is likely that reactive oxygen species, in addition to its well-known effect on NO availability, may play a role in the upregulation of the downstream enzyme sGC in the coronary microvessels, a hypothesis that has yet to be tested in future investigations. Furthermore, the questions that also remain to be answered are to what extent and how long the upregulation of sGC may contribute to the maintenance of NO-mediated arteriolar vasmotor responses to provide adequate perfusion of the myocardium during the disease development.

In summary, the findings of the present study suggest that in coronary arterioles of high-fat diet-induced obese rats, an increased activity of sGC leads to the enhanced sensitivity of smooth muscle cells to NO, a mechanism that may contribute to preserved coronary arteriolar dilations. We suggest that this mechanism could contribute to the early adaptation of coronary arterioles for providing adequate blood flow to meet the enhanced metabolic demand due to obesity.

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GRANTS
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