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Influence of Leptin on Arterial Distensibility

A Novel Link Between Obesity and Cardiovascular Disease?

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Background—The mechanisms by which obesity increases the risk of atherosclerotic cardiovascular disease (CVD) are poorly understood. In experimental models, leptin, a hormone produced by adipose tissue, has been shown adversely to affect vascular health. Therefore, we tested the hypothesis that high leptin concentrations are associated with lower arterial distensibility, an index of circulatory function relevant to the atherosclerotic process.

Methods and Results—Noninvasive, high-resolution, vascular ultrasound was used to measure brachial artery distensibility in 294 healthy adolescents (aged 13 to 16 years) who had a broad range of body mass indexes. Fat mass was measured by bioelectric impedance analysis; fasting serum leptin concentration by radioimmunoassay; and lipid profile, fasting insulin, glucose, and C-reactive protein concentrations by standard laboratory techniques. Higher leptin concentrations were associated with impaired arterial distensibility (regression coefficient, -1.3% change in arterial distension per 10% increase in leptin; 95% CI, -1.9% to -0.8% ; $P < 0.001$). This association was independent of fat mass, blood pressure, and C-reactive protein, fasting insulin, or LDL cholesterol concentrations.

Conclusions—Elevation in leptin was associated with impaired vascular function, independent of the metabolic and inflammatory disturbances associated with obesity. Our observations are consistent with data from experimental models and suggest that high leptin concentration is an important mechanism for the adverse influence of body fatness on CVD. (*Circulation*. 2002;106:1919-1924.)

Key Words: atherosclerosis ■ obesity ■ cardiovascular diseases

Obesity is a major risk factor for the development of atherosclerotic cardiovascular disease (CVD),¹⁻³ yet the mechanisms that relate fat mass to vascular health are only partially understood. Recently, obesity has been associated with lower arterial distensibility,⁴⁻⁷ a measure of the elastic properties of the vessel wall, and an index of circulatory function relevant to the atherosclerotic process.^{8,9} Furthermore, weight loss has been shown to improve arterial stiffness.^{4,10,11} It is not clear, however, whether obesity has a direct influence on arterial distensibility or whether its adverse effects are mediated by risk factors associated with obesity, such as insulin resistance^{6,12} or inflammation.

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The effect of obesity on vascular function may be mediated by the hormone leptin. Leptin is produced by adipocytes and plays a key role in the regulation of appetite and body weight.^{13,14} Leptin concentrations rise exponentially with increasing percentage body fat,¹⁴ and obese individuals have markedly increased leptin production, probably as a consequence of resistance to its actions.^{13,14} However, the wide-

spread distribution of functioning leptin receptors on vascular cells suggests that leptin also plays an important role in vascular physiology.¹⁵⁻²⁰ In experimental models, leptin has been shown to have angiogenic activity,^{15,16} increase oxidative stress in endothelial cells,¹⁷ and to promote vascular cell calcification¹⁹ and smooth muscle cell proliferation and migration.²⁰ Leptin's role linking fat mass and atherogenesis is supported by findings in the ob/ob mouse, which lacks a functioning leptin gene. These mice are resistant to atherosclerosis despite being grossly obese.^{21,22} Alternatively, obesity could influence vascular function by an effect on inflammation. C-reactive protein (CRP) is elevated in obese individuals and associated with a greater risk of CVD.

We have examined the association between leptin and arterial distensibility and its relation to the inflammatory and metabolic disturbances associated with obesity. We studied adolescents to permit evaluation of the role of leptin on vascular changes early in the development of arterial disease and without the presence of confounding risk factors present in an older population. Our subjects had a broad representative range of body fatness, thereby enabling us to compare the

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relative importance of leptin and adiposity on vascular function.

Methods

Subjects

The 294 adolescents studied (aged 13 to 16 years) were recruited from a preterm birth cohort (216 participants) and from schools in the same communities (78 born at term).^{23,24} All subjects were nonsmokers and clinically well. The subjects and their parents gave informed consent, and national and local research ethics committees approved the study.

Arterial Distensibility

Arterial distension was measured in the right brachial artery by high-resolution ultrasound²⁵ and a Wall Tracking System (Ingenious Medical Systems).²⁶ The mean change in diameter between diastole and systole over a 5-second period was measured. An average of 3 distension measurements were computed. Coefficients of variation for measurements of diameter and distension with this technique are reported as 2% to 3%.^{27,28} Blood pressure was measured in the left brachial artery with an automated device (Accutorrsat, Datascope Corp) during distension measurement in the right arm. This provided a representative measure of the pulse pressure in the right brachial artery, which was used to derive arterial distensibility from arterial distension.²⁵ Blood pressure was also determined before the measurement of arterial distensibility, and the mean of the 2 measurements was used to assess associations of mean arterial blood pressure with arterial distensibility.

Flow-Mediated Endothelial-Dependent Vasodilation

Flow-mediated endothelial-dependent vasodilation (FMD) was measured as described previously²⁹ and expressed as the maximal change between prehyperemic and posthyperemic brachial artery diameter as a percentage of prehyperemic diameter.²⁹

Anthropometric, Demographic, Fat Mass, and Biochemical Measurements

Body mass index (BMI) was calculated from height and weight measurements. Fat mass was determined by bioelectric impedance analysis (EZ COMP 1500, Fitness Concepts Inc) and skinfold thickness. Fat mass was obtained from the manufacturer's internal algorithm and also calculated with the equations of Schaefer,³⁰ which are suitable for adolescents. Triceps, biceps, and subscapular and suprailliac skinfold thicknesses were measured with the use of skinfold calipers. The standard deviation scores (z scores) for weight, height, and BMI were calculated according to reference growth data.

Blood was obtained by venepuncture between 9:00 AM and 11:00 AM after an overnight fast and serum stored at -80°C . Serum leptin concentrations were determined with the use of a commercially available radioimmunoassay (Linco Research Inc) with a detection limit of $0.5\ \mu\text{g/L}$ (intra-assay and interassay coefficient of variation of 2% and 5%, respectively). Fasting concentrations of CRP, insulin, and total, HDL, and LDL cholesterol were determined by standard laboratory methods.

Statistical Analysis

Multiple linear regression analysis was used to assess associations between arterial distensibility and leptin concentration. Arterial distensibility is usually represented as the distensibility coefficient calculated as the change in cross-sectional area between diastole and systole relative to the area at diastole divided by pulse pressure. However, to assess associations between arterial distensibility and explanatory variables, absolute arterial distension (mm) was used as the dependent variable and adjusted for pulse pressure by regression analysis as described previously.²⁵ Associations between arterial distension and explanatory variables were also adjusted for potential confounding factors (age, sex, and anthropometric variables—dia-

stolic arterial diameter and z scores for height and weight). Finally, stepwise regression analysis was used to identify independent associations of arterial distension.

Arterial distension and serum leptin concentration were not normally distributed and were \log_e -transformed and then multiplied by 100 before statistical analyses.³¹ Therefore, the standard deviation for \log_e leptin concentration multiplied by 100 represents the coefficient of variation, and regression coefficients for the 100 \log_e -transformed distension data represent the percentage change in arterial distension per unit change in independent variable.³¹ Thus, for regression analyses that included leptin concentration, the coefficients represent the percentage change in arterial distension per percentage change in serum leptin concentration (presented in the results as per 10% change in leptin concentration for simplicity). Statistical significance was taken as $P<0.05$ for all analyses with the use of two-tailed tests.

Results

Arterial distensibility did not differ significantly in adolescents born at term or before term (data not presented). Furthermore, there were no significant interactions between leptin concentration and preterm birth ($P=0.6$) or sex ($P=0.9$) on arterial distensibility. We therefore analyzed the combined population whose anthropometric, demographic, metabolic, and vascular characteristics are given in Table 1.

Arterial Distension and Fat Mass

Arterial distension was significantly associated with fat mass independent of potential confounding factors (pulse pressure, age, sex, z score for height and weight, and diastolic arterial diameter) (Table 2). This association remained significant after adjustment for potential confounding factors (as above) together with insulin concentration ($P=0.01$) or LDL cholesterol concentration ($P=0.02$). Arterial distension, however, was not related to BMI z score or to metabolic disturbances associated with obesity (Table 2).

Arterial Distension and Leptin

There was a strong inverse association between arterial distension and leptin concentration (regression coefficient, -1.3% change in arterial distension per 10% increase in leptin concentration; 95% CI, -1.9% to -0.8% ; $P<0.001$) (Figure), and this was independent of potential confounding factors (Table 2). Furthermore, this association remained significant after adjustment for confounding factors together with 3 different measures of fat mass: using EZ COMP analyzer (regression coefficient, -1.2% ; 95% CI, -2.3% to -0.1% ; $P=0.03$), using Schaefer's equations (regression coefficient, -1.2% ; 95% CI, -2.3% to -0.1% ; $P=0.03$), and using the sum of skinfolds (regression coefficient, -1.5% ; 95% CI, -2.7% to -0.3% ; $P=0.01$).

The association of arterial distension with leptin concentration was also independent of confounding factors together with the metabolic and inflammatory disturbances associated with obesity, including fasting insulin, LDL cholesterol, HDL cholesterol, and CRP concentration or mean arterial blood pressure (data not presented). As expected, fat mass was significantly correlated with CRP concentration ($r=0.20$, $P=0.001$) and fasting insulin concentration ($r=0.43$, $P<0.001$).

A stepwise multiple regression model was fitted with arterial distension as the dependent variable and pulse pres-

TABLE 1. Demographic, Metabolic, and Arterial Variables in 294 Subjects

Variable	All (n=294)*		Boys (n=134)		Girls (n=160)	
	Mean	SD	Mean	SD	Mean	SD
Age, y	14.9	0.9	15.0	0.9	14.9	0.9
Sex, No. males (%)	134	(46)
Weight, z score	0.1	1.2	0.2	1.1	0.1	1.2
Height, z score	-0.3	1.1	-0.1	1.1	-0.4	1.1
BMI, z score	0.4	1.2	0.4	1.2	0.4	1.2
Sum of skinfolds, mm†	51	38	33	30	59	29
Fat mass A, kg‡	9.7	10.0	7.6	8.0	12.6	10.7
Fat mass B, kg‡§	12.7	11.3	10.1	9.5	14.2	11.6
Waist/hip ratio	0.8	0.1	0.8	0.1	0.8	0.1
Leptin, $\mu\text{mol/L}$	5.7	100	2.8	80	10.2	70
Cholesterol, mmol/L	4.0	0.8	3.8	0.8	4.1	0.7
LDL cholesterol, mmol/L	2.6	0.7	2.5	0.8	2.7	0.6
HDL cholesterol, mmol/L	1.2	0.3	1.2	0.3	1.2	0.2
Triglycerides, mmol/L†	0.8	0.4	0.8	0.5	0.8	0.4
Insulin, pmol/L†	48.0	31.0	45.5	31.0	50.0	32.0
Glucose, mmol/L	4.7	0.5	4.8	0.4	4.6	0.5
CRP, mg/dL†	0.3	0.6	0.3	0.5	0.3	0.7
Blood pressure, mm Hg						
Diastolic	63.9	7.7	63.4	7.8	64.4	7.5
Systolic	115.9	8.9	117.7	8.6	114.2	8.7
Mean	84.5	7.4	84.8	7.3	84.3	7.4
Pulse	52.7	9.6	55.8	10.6	50.1	7.8
Arterial						
Diameter, mm	2.9	0.4	3.2	0.4	2.7	0.3
Distension, mm	0.11	46	0.13	41	0.10	46
Distensibility coefficient ($\times 10^{-3} \cdot \text{kPa}^{-1}$)	11.4	44	11.5	40	11.4	47
FMD, %	6.5	3.2	5.8	2.8	7.0	3.5
Reactive hyperemia, %	685	331	686	351	684	313

FMD indicates flow-mediated endothelial dependent vasodilation.

*Some loss of n for some variables.

†Median, interquartile range.

‡Fat mass according to EZ COMP analyzer.

§Fat mass according to equations of Schaefer.

||Geometric mean and coefficient of variation (%).

sure, potential confounding factors, mean arterial pressure, and variables in Table 2 as potential explanatory variables. The final model accounted for 24% of the variability in arterial distension. Arterial distension was significantly associated with pulse pressure, z score for weight, and mean arterial blood pressure, as expected, but was also significantly and independently associated with fasting leptin concentration (regression coefficient, -1.4% ; 95% CI, -1.9 to -0.8% ; $P < 0.001$) (Table 3).

FMD, Brachial Arterial Diameter, and Leptin

FMD was not significantly associated with leptin concentration ($P = 0.8$) even after adjustment for confounding factors. Similar observations were made for the association of brachial artery diameter with leptin concentration ($P = 0.2$).

Discussion

Obesity is associated with an increased risk of atherosclerotic CVD,¹⁻³ but the physiological link between fat mass and vascular disease is unexplained. Our study in healthy adolescents suggests a marked influence of leptin on arterial distensibility. This observation, together with evidence from experimental models¹⁵⁻²⁰ and from prospective studies that show an influence of leptin on clinical outcomes,^{32,33} strongly suggests that leptin is one mechanism by which body fatness is linked to the early stages of atherosclerosis.

The effect of obesity on vascular function in children and adolescents, rather than adults, has received little attention. Recently, Tounian et al⁷ showed that obese adolescents had greater arterial stiffness than lean controls. We studied

TABLE 2. Relations Between Arterial Distension and Leptin, Fat Mass, and Metabolic Variables

Variable	All (n=294)*			Boys (n=134)			Girls (n=160)		
	Regression Coefficient, %	95% CI, %	P	Regression Coefficient, %	95% CI, %	P	Regression Coefficient, %	95% CI, %	P
Leptin, per 10% change	-1.5	-2.5 to -0.6	0.003	-1.6	-3.0 to -0.3	0.02	-1.5	-3.0 to 0.2	0.08
Fat mass A, kg†	-1.2	-2.1 to -0.3	0.01	-1.6	-2.7 to -0.4	0.007	-0.6	-2.2 to 0.9	0.4
Fat mass B, kg‡	-1.7	-3.0 to -0.4	0.01	-1.9	-3.5 to -0.3	0.02	-1.6	-3.7 to 0.6	0.2
Sum of skinfolds, mm	-0.3	-0.6 to 0.0	0.08	-0.5	-0.9 to -0.1	0.01	-0.08	-0.6 to 0.4	0.8
Cholesterol, mmol/L	-5.8	-12.8 to 1.2	0.1	-9.3	-19.0 to 0.5	0.06	-0.8	-11.3 to 9.8	0.9
BMI, z score	21.2	-4.3 to 46.8	0.1	34.5	0.9 to 68.1	0.04	-4.4	-60.4 to 51.6	0.9
LDL cholesterol, mmol/L	-5.6	-13.6 to 1.8	0.1	-8.7	-18.7 to 1.3	0.09	-0.6	-12.3 to 11.2	0.9
CRP, mg/dL	-4.6	-13.7 to 4.5	0.3	-4.5	-17.8 to 9.0	0.5	-5.1	-18.0 to 7.8	0.4
HDL cholesterol, mmol/L	-7.6	-27.2 to 12.0	0.5	0.9	-24.6 to 26.4	0.9	-10.6	-42.7 to 21.4	0.5
Insulin, pmol/L	-0.04	-0.2 to 0.1	0.5	-0.3	-0.6 to 0.0	0.03	0.02	-0.1 to 0.2	0.8
Triglycerides, mmol/L	-1.4	-11.4 to 8.6	0.8	-13.0	-28.2 to 2.5	0.1	5.1	-8.5 to 18.7	0.5
Glucose, mmol/L	-0.6	-11.2 to 10.0	0.9	-0.1	-16.0 to 15.7	0.9	-0.09	-15.3 to 13.5	0.9
Waist/hip ratio	0.9	-46.3 to 48.1	0.9	-36.8	-97.9 to 24.3	0.2	31.8	-43.7 to 107.0	0.4

Each line represents a separate regression model. Regression coefficients are % difference in arterial distension per unit change in independent variable. All models are adjusted for pulse pressure, age, z score for height and weight, and diastolic arterial diameter, and the combined group is also adjusted for sex.

*Some loss of n in some models.

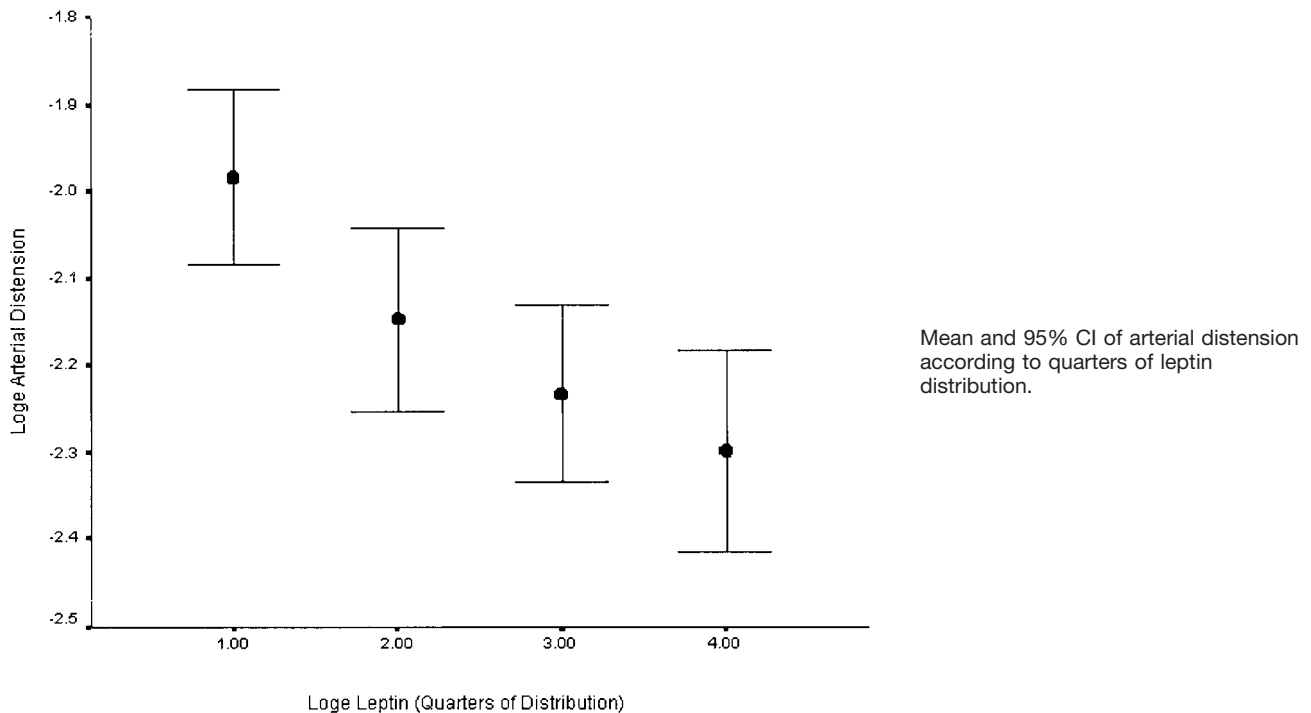
†Fat mass according to EZ COMP analyzer.

‡Fat mass according to equations of Schaefer.

children who did not have potential confounding factors often found in older populations, such as hypertension, dyslipidemia, and insulin resistance.^{4-6,10,11} Furthermore, unlike the earlier report,⁷ our findings were not confined to obese individuals and suggest that even moderate degrees of body fatness influence structural changes related to atherosclerotic disease progression from a young age.

Experimental work suggests several mechanisms by which leptin may affect arterial disease.¹⁵⁻²⁰ Although predominantly involved in the hypothalamic control of body weight, leptin receptors are widely distributed on endothelial cells,¹⁵⁻¹⁷ on other arterial wall subpopulations,¹⁹ and on atherosclerotic lesions.^{18,19} An action of leptin via these receptors may stimulate smooth muscle cell proliferation and migration,²⁰ whereas prolonged treatment with leptin has been shown to increase vascular cell calcification.¹⁹ Leptin has also been shown to induce oxidative stress in endothelial cells, which could contribute to vascular pathology.¹⁷ Furthermore, a direct influence of leptin on vascular health is supported by the ob/ob mouse, which lacks leptin and consequently becomes hyperphagic and grossly obese but is nevertheless resistant to atherosclerosis.^{21,22} Atherosclerosis risk in heterozygotes is intermediate between ob/ob homozygotes and control animals, which suggests a dose-response relation between leptin levels and the atherosclerotic process.²² Obese humans have increased leptin production per unit of fat mass¹³ and consequently disproportionately elevated leptin concentrations.¹⁴

We measured arterial distensibility, which is known to correlate closely with atherosclerotic risk factors^{4-7,10-12} (even from a young age^{7,25}), extent of disease,^{8,9} and cardiovascular risk.³⁴ The association of arterial distensibility with leptin was independent of fat mass and metabolic and inflammatory markers. Although we confirmed earlier studies that showed a relation between body fatness and CRP concentration or insulin resistance, the influence of leptin on arterial distensibility was not dependent on these variables. Insulin resistance has been suggested to explain the link between arterial distensibility and obesity.^{6,12} These studies, however, did not usually assess healthy children with a low risk of insulin resistance^{4-7,10-12} and often used BMI as a relatively inaccurate proxy for adiposity.⁶ BMI is a measure of both lean and fat mass,³⁵ and therefore inaccuracies in predicting fat mass from BMI could explain the earlier inconsistent associations between arterial distensibility and BMI^{4-6,10,12} and the lack of such a relation in our study. The influence of leptin was also independent of HDL concentration, even though higher HDL concentration has been postulated to mediate some of the beneficial vascular effects of leanness⁷ and the resistance to atherosclerosis in the ob/ob mouse.²² Our findings were not explained by blood pressure even though a fall in blood pressure has been proposed as an explanation for the observed improvement in arterial distensibility after weight loss in obese adults.^{10,11} Hormonal changes associated with greater adiposity such as higher cortisol concentrations are unlikely to explain our findings, because the association of arterial distension with leptin was



independent of fat mass. Finally, the lack of any influence of leptin concentration on brachial artery FMD suggests that the effect of leptin on arterial distensibility was independent of the short-term bioavailability of endothelial nitric oxide. This finding was not unexpected. Evidence from animal models suggests that leptin affects, adversely, arterial structural components such as vascular cell calcification¹⁹ and smooth muscle cell proliferation and migration (which is suggested to play a role in the intimal thickening of atherosclerosis).²⁰ Long-term exposure to high leptin concentrations, therefore, is more likely to affect arterial distensibility than FMD.

Potential Limitations

Although our study population had a higher proportion of subjects born before term than the normal population, all were clinically well, and arterial distensibility did not differ between those born at term and preterm. Moreover, anthropometric measures and social class profile were representative of the national population, and mean arterial distension (0.11 mm) was similar to that in a representative population of younger children (0.13 mm).²⁵ It seems likely, therefore, that the association between leptin and arterial distensibility can be extrapolated to other cohorts.

TABLE 3. Stepwise Regression Model of Arterial Distension on Explanatory Variables (n=254)

Variable	Regression Coefficient, %	95% CI, %	P
Pulse pressure, mm Hg	1.3	0.8 to 1.9	<0.001
Weight, z score	9.6	4.7 to 14.6	<0.001
Mean arterial pressure, mm Hg	-1.0	-1.7 to -0.3	0.006
Leptin (10%)	-1.4	-1.9 to -0.8	<0.001

Regression coefficients are % change in arterial distension per unit change in independent variable.

Our study does not prove a causal association between leptin concentration and vascular health, because this would require an experimental intervention. However, such a study would be difficult to do in humans and would be unable to investigate the effects of chronic hyperleptinemia on vascular function. Nevertheless, our hypothesis that leptin has an independent influence on vascular health is supported by recent prospective observations that showed that leptin is an independent risk factor for coronary events.^{32,33} These observations, together with our findings at an earlier stage of the atherosclerotic process, suggest that the effect of leptin on arterial health is clinically relevant and is not just an epiphenomenon.

Implications

The effect of high leptin concentration on vascular health has potentially important implications for understanding the role of obesity in the atherosclerotic process. Childhood obesity has been shown to increase the risk of later CVD.^{2,3} This effect has been explained previously by a prolonged exposure to the metabolic milieu associated with obesity (such as high insulin concentration³). It could equally be attributed, in part, to prolonged hyperleptinemia. Our finding of an association between leptin and vascular function in nonobese children raises the possibility that preventing even moderate obesity in childhood may have a long-term benefit for the risk of CVD that is independent of the adverse metabolic consequences of obesity.

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