Erciyes Med J 2022; 44(3): 324–9 • DOI: 10.14744/etd.2021.64249
ORIGINAL ARTICLE – OPEN ACCESS

Leptin Prevents U46619- and Angiotensin II-Elicited Contraction in Isolated Human Umbilical Vessels

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ABSTRACT

Objective: This study investigated the vasoactive responses of quiescent and pre-contracted isolated human umbilical veins and arteries to cumulative leptin.

Materials and Methods: The vasoactive response of umbilical vessels pre-contracted with U46619 (10^{-10}M) and angiotensin II (10^{-6}M) to cumulative leptin (10^{-11}-10^{-7}M) were recorded *in vitro*.

Results: Leptin did not affect the artery or vein basal tonus (p=1). The leptin-elicited relaxation in U46619-contracted vessels was greater in veins than in arteries (p<0.001). Incubation with N (omega)-nitro-L-arginine methyl ester (L-NAME) (10^{-4}M) prevented the leptin-induced relaxation of the U46619 contraction response in the artery. The E_{max} and pD_2 values of the L-NAME-incubated veins were lower than those of the non-incubated veins (p<0.001 and p=0.001, respectively). The relaxation in angiotensin II-contracted veins was greater than that of the arteries (p<0.001). Incubation with L-NAME prevented leptin-induced relaxation in angiotensin II-contracted arteries. The E_{max} of leptin-induced relaxation of L-NAME-incubated veins was significantly lower than that of non-incubated veins (p=0.027); the pD_2 was similar.

Conclusion: Leptin did not alter the resting tension of isolated umbilical vessels; however, the results indicated that leptin caused concentration-dependent relaxation in umbilical vessels pre-contracted with U46619 or angiotensin II. The maximum relaxation was greater in veins compared with arteries. Incubation with L-NAME completely inhibited leptin-induced relaxation in arteries and resulted in a significant inhibition in veins.

Keywords: Angiotensin II, leptin, U46619, umbilical artery, umbilical vein

Cite this article as:
Duman İ, Soner BC, İnan SY, Şahin AS. Leptin Prevents U46619- and Angiotensin II-Elicited Contraction in Isolated Human Umbilical Vessels. Erciyes Med J 2022; 44(3): 324-9.

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Submitted
21.06.2021

Accepted
17.11.2021

Available Online
20.04.2022

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INTRODUCTION

Leptin is a peptide hormone that regulates neuropeptide production in several areas involved in food intake and energy expenditure in the central nervous system (1). Leptin binds to specific receptors in hypothalamic nuclei, thereby reducing appetite and stimulating energy use. Due to the wide distribution of leptin receptors in the body, leptin may also play a role in controlling immune, reproductive, and cardiovascular functions (2). Peripheral effects include reduced pancreatic insulin secretion, increased diuresis, and regulation of vascular tone (3, 4).

Leptin is produced mainly in adipose tissue. However, small amounts of leptin are also secreted from tissues in areas such as the liver, breast, bowel, skeletal muscle, and placenta (3). Serum leptin levels rise during pregnancy due to the increase in body fat and release of leptin from the placenta (5). The leptin receptor (OB-Rb) is produced in human vascular endothelium and mediates leptin-dependent angiogenic activity (6, 7). Although leptin stimulates the sympathetic nervous system, the vasodilation caused by leptin attenuates the effect of sympathetic discharge on blood pressure (8). The effect of leptin on vasoreactivity varies according to the species and type of vessel (9–11). The umbilical cord connected to the placenta is a conduit for blood and nutrients to the fetus. Endogenous or exogenous vasoactive substances are essential to control of fetoplacental circulation. The current study was designed to investigate the vasoactive response of quiescent and pre-contracted isolated human umbilical veins and arteries to cumulative leptin.

MATERIALS and METHODS

This study was approved by the Necmettin Erbakan University, Meram Faculty of Medicine Ethics Committee (date: 06.01.2017, number: 783) and designed according to EQUATOR (Enhancing the Quality and Transparency of Health Research) Network guidelines (12). Umbilical cord samples of remnant tissue that would otherwise be discarded were obtained with maternal consent from full-term vaginal deliveries.

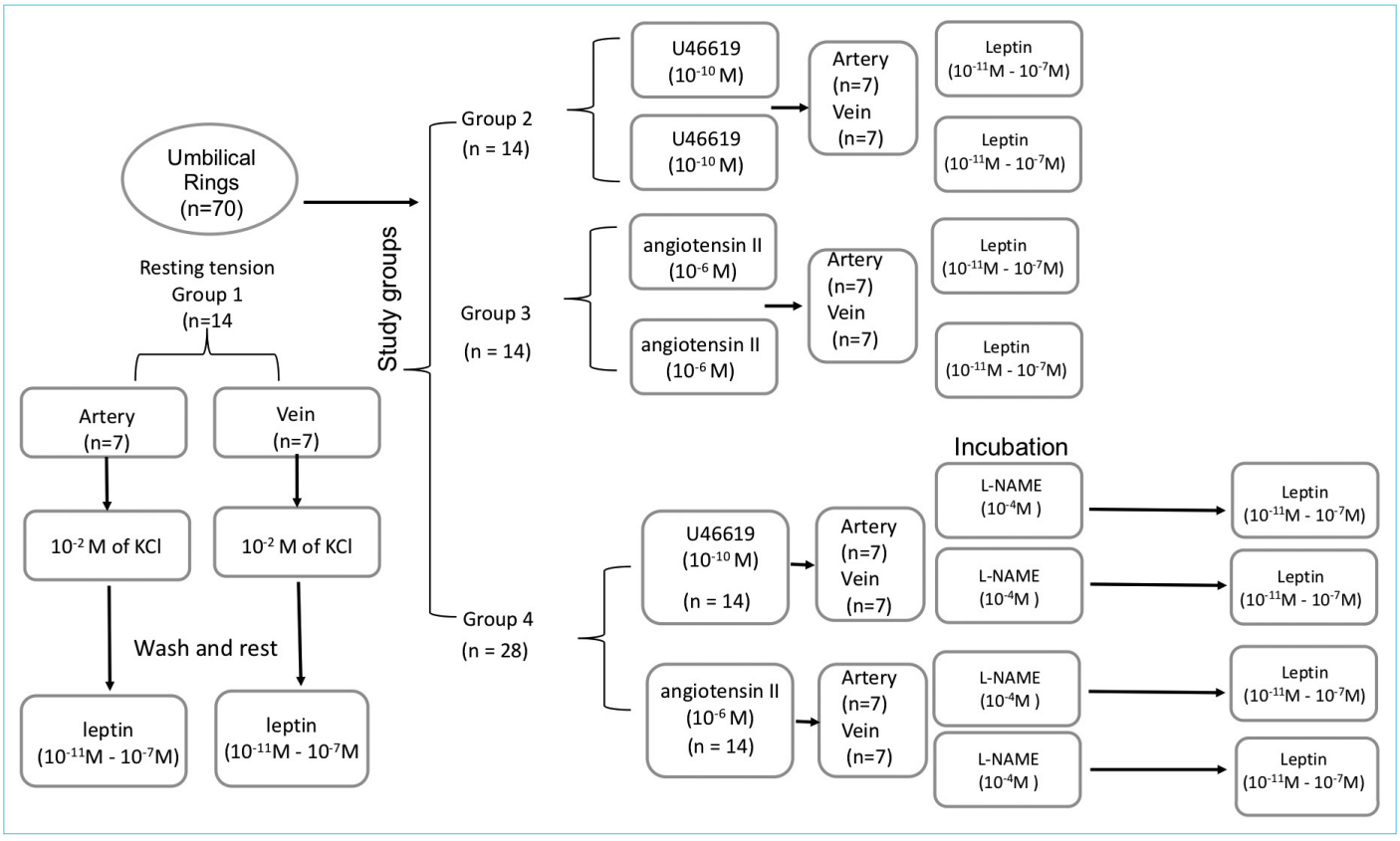

Figure 1. Flow chart of the experimental procedures

Tissue Preparation

Within 10 minutes of delivery, a segment of untouched umbilical cord was carefully resected from the placental side. The cord was transported immediately to the laboratory in cold Krebs-Henseleit solution (KHS). The umbilical arteries and veins were then meticulously separated from the surrounding tissue and the isolated vessels were cut into ring segments of 3–4 mm in length.

The umbilical rings were then suspended between 2 stainless steel hooks in an organ bath containing 10 mL of KHS buffer, which was gassed with 95% O_2 - 5% CO_2 and maintained at 37oC as previously described (13). One steel hook was connected to the organ bath (Isolated tissue bath - IOBS99, Commat Ltd., Ankara, Turkey), and the other was connected to a force transducer (MP36; Biopac Inc., Goleta, CA, USA) to continuously record isometric tension on a computer.

The tissue rings were randomized into 4 primary study groups, and each ring was used only once. Before administering vasoactive drugs, the rings were stretched to an initial resting tension of 1 g and allowed to equilibrate for 60 minutes. The buffer was changed every 15 minutes. After the stabilization period, the experiments were performed, as summarized in Figure 1.

Experimental Procedures

Group 1- Effect of leptin on the resting tension of the umbilical artery and vein

The vessels were initially exposed to 10^{-2} M of KCl to measure contractility. After a washout and resting period, cumulative leptin (10^{-11}-10^{-7}M) was added to the bath and the concentration-response curve of effect on the basal tonus of the resting umbilical artery (n=7) and vein (n=7) rings was recorded.

In subsequent experiments, 2 potent vasoconstrictor agents with specific modes of action were used to elicit contractions on umbilical vessels. U46619 (9,11-dideoxy-11alpha,9alpha-epoxymethanoprostaglandin F2alpha) is a thromboxane A_2 (TXA_2)/T prostanoid (TP) receptor agonist, and angiotensin II is a peptide that is part of the renin-angiotensin-aldosterone system, which regulates blood pressure and fluid as well as electrolyte balance and systemic vascular resistance.

Group 2- Effect of leptin on U46619-elicited contraction

The maximum contraction response with U46619 (10^{-10}M) in the umbilical artery (n=7) and vein (n=7) rings was obtained at resting tension. Then, when the contraction reached its maximum level, the concentration-response curve was recorded by adding cumulative leptin (10^{-11}M - 10^{-7}M) to the organ bath.

Group 3- Effect of leptin on angiotensin II-elicited contraction

As in the previous group, the maximum contraction response with angiotensin II (10^{-6}M) in the umbilical artery (n=7) and vein (n=7) rings was obtained at resting tension. When the contraction reached its maximum level, the concentration-response curve was recorded by adding cumulative leptin (10^{-11}M - 10^{-7}M) to the organ bath.

Group 4- Role of nitric oxide (NO) in leptin elicited relaxation

Once the maximum contraction response with U46619 (10^{-10}M) was recorded, nitric oxide (NO) synthesis was blocked by incubating the umbilical artery (n=7) and vein (n=7) rings with NO synthase

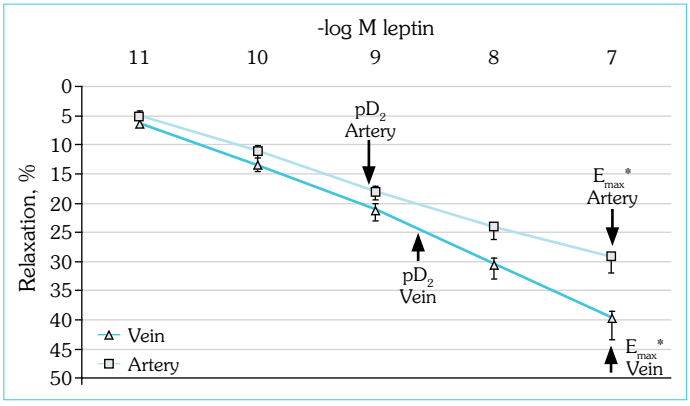

Figure 2. Relaxation response of cumulative leptin (10^{-11}-10^{-7}M) on maximum contraction response of U46619 (10^{-10}M) in umbilical artery and umbilical vein rings. Data are presented as the percent relaxation of the maximum contraction response of U46619

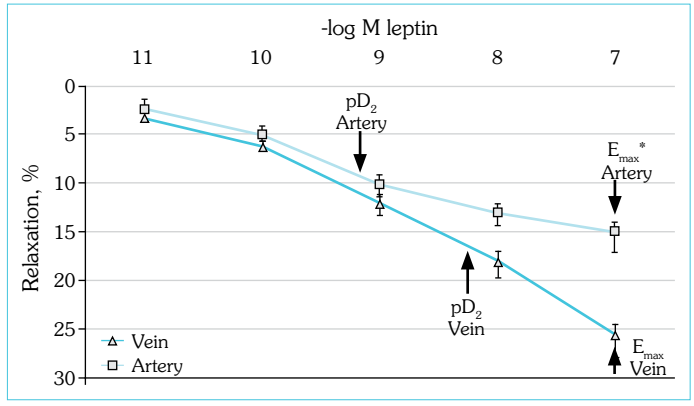

Figure 3. Relaxation response of cumulative leptin (10^{-11}-10^{-7}M) on maximum contraction response of angiotensin II (10^{-6}M) in umbilical artery and umbilical vein rings. Data are presented as the percent relaxation of the maximum contraction response of angiotensin II

(NOS) inhibitor N (omega)-nitro-L-arginine methyl ester (L-NAME) (10^{-4}M) for 15 minutes and the concentration-response curve of cumulative leptin (10^{-11}-10^{-7}M) was recorded.

The maximum contraction evoked by the vasoconstrictor agents U46619 and angiotensin II was considered the baseline for subsequent relaxation. Relaxation was expressed as the percentage of reduction in vasoconstrictor-elicited contraction.

Drugs and Chemicals

The composition of KHS was [mM]: NaCl 118.3; KCl 4.69; KH_2PO_4 1.18; $CaCl_2$ 1.25; $MgSO_4$ 1.17; $NaHCO_3$ 25.0; glucose 11.1; pH:7.4. The human recombinant leptin, angiotensin II, and L-NAME used in the study were purchased from Sigma-Aldrich (MilliporeSigma, St. Louis, MO, USA) and U46619 was obtained from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA). All concentrations were expressed as molar concentrations (M).

Statistical Analysis

The study data were analyzed using SPSS Statistics for Windows, Version 17.0 software (SPSS Inc., Chicago, IL, USA). In addition, Excel for Mac 2018 (Microsoft Corp. Redmond, WA, USA) was used to create the figures. Leptin-induced relaxation responses were evaluated as the percentage of the maximum contraction response of the contraction agent used (10^{-10} U46619; 10^{-6}M angiotensin II). E_{max} (% maximum relaxation) and pD_2 (negative logarithm of the concentration, which decreases the maximum effect of an agonist by 50%) values were calculated for leptin. The Shapiro-Wilk test was used to assess the normality of the numerical variables. Arithmetic mean±SD was used to summarize the data. Since the distribution of the data was normal, the Student t-test was used to compare independent groups. A p value of <0.05 was considered statistically significant.

RESULTS

There was no change in the initial resting tension (basal tonus) of the umbilical artery and vein rings after administration of cumulative leptin (10^{-11}-10^{-7}M) (p=1).

Table 1. The calculated E_{max} and pD_2 values of leptin (10^{-11}-10^{-7}M)-induced relaxation on human umbilical arteries and veins contracted with U46619 (10^{-10}M) and effects of incubation with L-NAME (10^{-6}M)

	U46619 (N=28)		
	L-NAME -	**L-NAME +**	**p**
E_{max}			
Artery	29.29±2.56 (n=7)	NC (n=7)	N/A
Vein	39.57±3.78 (n=7)	19.29±2.87 (n=7)	<0.001
p	<0.001	N/A	
pD_2			
Artery	9.056±0.26 (n=7)	NC (n=7)	N/A
Vein	8.70±0.40 (n=7)	7.65±0.47 (n=7)	0.001
p	0.081	N/A	

Data are presented as mean±SD; E_{max}: (%); pD_2: -Log. L-NAME: N (omega)-nitro-L-arginine methyl ester; N/A: Not applicable; NC: Not calculated due to complete inhibition of relaxation

As illustrated in Figure 2, cumulative leptin (10^{-11}-10^{-7}M) induced a concentration-dependent relaxation response after the maximum contraction response of U46619 (10^{-10}M) in both the artery and vein rings. The observed vasodilatation was more pronounced in veins when compared with arteries. The E_{max} of leptin was significantly higher in the umbilical vein (39.57±3.78%) than the artery (29.29±2.56%) (p<0.001), while the pD_2 of leptin was similar in the artery (9.05±0.26 -log EC_{50}) and vein (8.70±0.40 -log EC_{50}) rings (p=0.081). Incubation with L-NAME (10^{-4}M) prevented cumulative leptin-induced relaxation after maximum contraction response with U46619 in arteries. The E_{max} and pD_2 of the L-NAME- incubated veins were significantly lower than those of the non-incubated veins (p<0.001 and p=0.001 respectively) (Table 1).

Table 2. The calculated E_{max} and pD_2 values of leptin (10^{-11}-10^{-7}M)-induced relaxation on human umbilical arteries and veins contracted with angiotensin II (10^{-6}M) and effects of incubation with L-NAME (10^{-6}M)

	Angiotensin II (N=28)		
	L-NAME -	L-NAME +	p
E_{max}			
Artery	15.00±2.16 (n=7)	NC (n=7)	N/A
Vein	25.57±2.37 (n=7)	21.29±3.08 (n=7)	0.027
p	<0.001	N/A	
pD_2			
Artery	9.25±0.43 (n=7)	NC (n=7)	N/A
Vein	8.33±0.43 (n=7)	8.41±0.34 (n=7)	0.73
p	0.002	N/A	

Data are presented as mean±SD; E_{max}: (%); pD_2: -Log. L-NAME: N (omega)-nitro-L-arginine methyl ester; N/A: Not applicable; NC: Not calculated due to complete inhibition of relaxation

Figure 3 illustrates the concentration-dependent relaxation response induced by cumulative leptin (10^{-11}M - 10^{-7}M) on the maximum contraction response of angiotensin II (10^{-6}M) in both artery and vein rings. The vasodilation effect elicited by leptin was significantly greater in the veins than the arteries. The E_{max} of leptin was significantly lower in the umbilical arteries (15.00±2.16%) than the veins (25.57±2.37%) ($p<0.001$), and the pD_2 was significantly greater in the arteries (9.25±0.43 -log EC_{50}) than the veins (8.33±0.43 -log EC_{50}) ($p=0.002$), suggesting that the potency of leptin for EC_{50} (half-maximal effective concentration) is greater in the arteries than the veins. Incubation with L-NAME (10^{-4}M) prevented cumulative leptin-induced relaxation after maximum contraction response with angiotensin II in arteries. The E_{max} of leptin-induced relaxation of L-NAME-incubated veins was significantly lower than that of non-incubated veins ($p=0.027$), while the pD_2 was similar ($p=0.73$) (Table 2).

DISCUSSION

Leptin is a fat-derived adipokine that has an important role in regulating body weight. Obesity, metabolic syndrome, and pregnancy lead to an increase in plasma leptin levels (3, 5). During pregnancy, leptin is produced in the fetoplacental unit, resulting in plasma leptin concentrations that are 2-fold higher than in the non-gravid state. A dysregulation of leptin production has been associated with alterations in fetal growth in several pregnancy pathologies (5). In addition, umbilical vessels do not have a vasa vasorum; therefore, they depend on their own oxygen supply, making them susceptible to changes in maternal circulation, such as hypertension (14). Human placental vessels have no autonomous innervation; therefore, vasoactive substances in maternal circulation are crucial for regulating umbilical-placental circulation and fetus development (15). Hypertension, which increases the risk of cardiovascular and cerebrovascular diseases, is the most common risk factor during pregnancy and both maternal and fetal consequences (3, 16).

In addition to other vasoactive substances, angiotensin II and prostanoids are involved in the pathophysiology of hypertension in pregnancy (17, 18). The maternal infusion of angiotensin II in sheep was reported to increase maternal arterial pressure and uterine vascular resistance and decrease the blood flow to the uterus (19). TXA_2 synthase and the TP receptor gene are present in the human uterus and uterine arteries (20). U46619, a stable PGH_2 analog, interacts with the TXA_2 receptor/TP receptor and has been used in other experimental research (21). *In vitro* studies showed that prostanoids, especially TXA_2, may be involved in modulating intrauterine blood flow and play a role in many cellular processes, including vascular homeostasis (20).

Changes in leptin levels may affect fetal circulation in hypertensive pregnancies. Our study findings indicated that leptin did not affect the tonus of quiescent umbilical vessels, but caused significant concentration-dependent relaxation in the pre-contracted umbilical arteries and veins. This relaxation is vital in umbilical veins, which carry oxygenated, nutrient-rich blood from the placenta to the fetus. Lepercq et al. (22) observed an increase in placental leptin levels in patients with severe preeclampsia. This increase in serum leptin was associated with an increase in the umbilical artery resistance index and may have been induced by the need for relaxation in the umbilical artery. Leptin also increases blood supply to the placenta by inducing neovascularization and is involved in supplying nutrients to an under-perfused placenta (5).

Our results with umbilical vessels are partially consistent with previous *in vitro* reports of studies of various vessels. Leptin has been reported to cause concentration-dependent vasodilation in norepinephrine-elicited contractions human internal mammary artery rings (23), a rat aorta pre-contracted with phenylephrine (8) and angiotensin II (24), and canine mesenteric arteries pre-contracted with noradrenaline (11).

The results of the present study also show that leptin was associated with significant concentration-dependent relaxation in pre-contracted umbilical artery and vein rings. The pD_2 values of U46619 revealed no significant difference in the potency of leptin on arteries or veins. However, the maximum relaxant effect was greater in umbilical veins than in arteries. Although leptin was more potent in terms of the EC_{50} on angiotensin-II-elicited contraction in umbilical arteries, it also caused more relaxation at the maximum concentration in the umbilical veins than in umbilical arteries. The difference may have been because the relaxation in umbilical arteries was completely NO-mediated, whereas, in veins, other mechanisms were involved in addition to NO-mediated mechanisms of relaxation caused by leptin. This NO-mediated relaxation in arteries is consistent with previous reports. NO is formed from L-arginine by the NOS enzyme in endothelial cells. This highly lipophilic substance prompts relaxation by activating the guanylate cyclase-cyclic guanosine monophosphate pathway in the smooth muscle (25). The critical role of NO in blood pressure regulation is well known. Endothelial NOS, in addition to producing NO and providing vasorelaxation via the vascular smooth muscle, also inhibits proliferation of vascular smooth muscle, infiltration of inflammatory cells, and platelet aggregation (6).

The presence of leptin receptors has been demonstrated in vascular smooth muscle and endothelial cells (7). NO-mediated vasodilation with leptin was observed in an *in vitro* study of rabbit aorta rings (10). It was noted in another study of isolated rat aorta that damage to the endothelium eliminated the phenylephrine-induced relaxation response to leptin, and that L-NAME inhibited leptin-induced relaxation responses (9). Similarly, NO-dependent relaxation responses were reported in rat mesenteric arteries contracted with phenylephrine (26).

The mechanisms underlying relaxation in the umbilical vein are complex. In a study on isolated human saphenous vein rings, Momin et al. (27) found that the leptin-induced dilatation was not NO-dependent. In contrast, in another study conducted using spontaneously hypertensive rats, cumulatively applied leptin caused contraction in the thoracic aorta and the pulmonary artery (28). This disparity might be explained by structural differences in the endothelium of different vascular beds. Intra-arterial leptin infusion to the forearm circulation of humans resulted in a significant increase in forearm blood flow and a significant decrease in forearm vascular resistance whether or not a NOS inhibitor was present, suggesting that leptin causes vasodilation by a NO-independent mechanism (29). Lembo et al. (8) investigated the mechanisms of the vasorelaxant action of leptin. They found that leptin-induced vasodilatation was inhibited by L-NAME and ODQ, a soluble guanylyl cyclase inhibitor that specifically inhibits endothelial NO function. In contrast, the vasorelaxant effect of leptin on mesenteric arteries was inhibited by Brefeldin A, an EDHF inhibitor, but was not influenced by NO. The authors indicated that the mechanisms involved in the vasorelaxant action of leptin are heterogeneous and related to the vascular structure (8). Endothelium differences may explain the discrepancy in NO-mediation in the umbilical arteries and veins in the present study.

Leptin-mediated NO is also responsible for the inhibition of vasoconstriction induced by angiotensin II. It has previously been demonstrated that an increase in NO, mediated by leptin, inhibits the vasoconstriction induced by angiotensin II through the attenuation of the angiotensin II-induced increase of cytosolic calcium, thus resulting in vasodilation (6, 24).

Leptin also has different vasoactive effects on quiescent vessels. In the present study, when applied cumulatively, leptin did not change the resting tension of human umbilical arteries and veins. Similarly, it was reported that leptin did not significantly affect the basal tonus of isolated human internal mammary artery rings (23). Furthermore, leptin infusion was not determined to have direct vasodilator effects *in vivo* in the hindlimb vascular bed of conscious rats (30). However, Lembo et al. (8) demonstrated that leptin reduced the basal tonus of rat mesenteric arteries.

Despite the above-mentioned direct effects of leptin on the vasculature, leptin has no acute effect on blood pressure under normal clinical conditions. The biphasic effect of leptin on blood pressure regulation is balanced by activating both the sympathetic nervous system, which causes vasoconstriction, and depressor mechanisms (vasodilation and naturesis) (6, 26). Leptin is also accepted as an essential factor in the maintenance of vascular homeostasis and wall integrity (6).

Since the increase in vasoconstrictor substances, including angiotensin II and TXA_2, lead to marked vasoconstrictor responses in umbilical circulation and affect placental blood flows *in vivo*, the vasodilatory effect of leptin on constriction elicited by these potent vasoconstrictors may have clinical relevance. Our results also support previous findings in different vascular beds that NO plays a significant role in the vasodilatory properties of leptin (6).

Limitations

Limitations to this study include the fact that we only studied the effect of 2 potent vasoconstrictors and NO, which is the most crucial relaxation pathway, at clinically relevant leptin concentrations. The vasospasm of the umbilical vasculature is multifactorial. Therefore, it is not possible to investigate all mechanisms of leptin-induced relaxation with 2 vasoconstrictor agents. Also, our relatively small sample size may be considered a limitation; however, it was based on similar *in vitro* studies, which also employed 6–8 isolated tissue specimens for each group of experiments. The restriction in sample size is due to both ethical regulations and experimental difficulties that forced us and other authors to use a small number of samples in other *in vivo* and *in vitro* studies (8–10). Nevertheless, illustrating the potential relaxant effect of leptin on the umbilical arteries and veins provides additional information on the effects of the serum leptin level increases observed during pregnancy.

CONCLUSION

The results of the present study show that leptin caused concentration-dependent relaxation in umbilical vessels pre-contracted with vasoconstrictor agents U46619 and angiotensin II. However, leptin did not alter the resting tension of the isolated human umbilical vessels. The relaxation was more significant in the umbilical veins compared with arteries. Incubation with NO synthesis inhibitor L-NAME completely inhibited the leptin-induced relaxation in arteries and resulted in significant inhibition of leptin-induced relaxation responses in veins. These findings suggest that leptin-induced relaxation in the umbilical arteries is completely NO-mediated, though NO-independent relaxation mechanisms are also involved in the umbilical veins. Leptin may offer preventive effects under conditions of increased vascular resistance in the umbilical vessels during pregnancy.

Ethics Committee Approval: Necmettin Erbakan University, Meram Faculty of Medicine Ethics Committee granted approval for this study (date: 06.01.2017, number: 783)

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – İD, BCS, SYİ, ASŞ; Design – İD, BCS, SYİ, ASŞ; Supervision – İD, BCS, SYİ, ASŞ; Resource – İD, ASŞ; Materials – İD, SYİ, ASŞ; Data Collection and/or Processing – İD, ASŞ; Analysis and/or Interpretation – İD, BCS, SYİ, ASŞ; Literature Search – İD, BCS; Writing – İD, BCS, SYİ, ASŞ; Critical Reviews – İD, BCS, SYİ, ASŞ.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: Necmettin Erbakan University, Scientific Research Project Coordinator, funded the study. Grant number: 161218024.

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