

Effect of Intra-Venous Versus Intra-Arterial Leptin Infusion on Blood Pressure and Heart Rate

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Abstract: Objectives: In this study the effects of different Leptin concentrations on the blood pressure and heart rate *in vivo* in anesthetized rabbits were studied.

Methods: Sixty Rabbits were divided into two groups, first group received Leptin intra-venously and other group received Leptin intra-arterially. Blood pressure and heart rate in were recorded before and after administration of Leptin.

Results: A significant increase in mean arterial blood pressure (MABP) was seen after an intra-arterial injection of 3, 5 and 7 $\mu\text{g}/\text{kg}$ of Leptin. This increase in MABP was monitored for different durations 10, 20 and 30 minutes after the infusion of each Leptin concentrations. The trend of the increase in MABP with time was demonstrated with all three concentrations. Intra-venous infusion of Leptin caused a significant decrease in MABP after 10 minutes as well as after 20 and 30 minutes, with all three concentrations (3, 5, and 7 $\mu\text{g}/\text{kg}$). Heart rate (HR) was not changed significantly at the end of 30 min of infusion.

Conclusion: this *in vivo* study demonstrated that intra-venous Leptin infusion has a different effect on the MABP compared with intra-arterial infusion and this difference might be due to the site of action of Leptin.

Keywords: Leptin, cardiovascular, blood pressure, heart rate.

INTRODUCTION

Leptin is a polypeptide hormone synthesized in white adipose tissues and encoded by the *ob* gene [1]. It plays an important role in the energy balance. This Leptin effect is taking place by its interaction with specific hypothalamic receptors affecting appetite and satiety centers of the brain in a feedback loop [2-4]. Administration of Leptin to mice causes a decrease in food intake, weight loss and an increase in energy expenditure [5-7]. Leptin effects on the cardiovascular system include elevation in blood pressure [8] and vasodilatation [9]. For example, the increase in blood pressure might be caused by increasing the heart rate and vasoconstriction through the sympathetic effect of Leptin [8,10-12]. In contrast, the vasodilatation effect of Leptin modulated through a local mechanism involving nitric oxide (NO) release [9,13-15]. Recent study by Mohamad *et al.*, on the effect of Leptin on isolated rat mesenteric arteries demonstrated that, the administration of Leptin induces relaxation of vascular smooth muscle through an endothelium-dependent release of nitric oxide (EDNO). In the same study, they also showed that the vascular effect was abolished by

administration of NO synthase blocker L-nitro-L-arginine methyl ester (L-NAME) [16]. A significant correlation between blood pressure and plasma Leptin concentration in patients with essential hypertension was demonstrated [17]. It was also demonstrated that high plasma Leptin concentration might be involved in the development of cardiovascular diseases including myocardial infarction in obese people [18]. In animal module, Shek *et al.* had shown that chronic administration of high Leptin both intra-arterially or intravenously causes increased in blood pressure and heart rate [19].

The aim of this study was to find the effect of different Leptin concentrations on the cardiovascular system (heart rate and blood pressure) *in vivo* in anesthetized rabbits. Using different concentrations of Leptin infused through the veins and arteries.

METHODS

This study was approved by scientific research committee at Jordan University of Science and Technology and ethical approval by medical faculty research committee. Sixty New Zealand white adult rabbits of either sex, weight ranged between 1.5 and 2.5Kg were used in this study. Rabbits were divided into two groups (A and B), each group was consisting of thirty rabbits. Leptin was administered intra-arterially in rabbits of group A (three subgroups; A1, A2, A3 each consists of 10 animals and each one received different leptin concentration) while it infused intra-venously in rab-

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bits of group B (three subgroups; B1, B2, B3 consisting 10 animals each which received different leptin concentration). Rabbits, in the beginning of each experiment, were anesthetized with sodium pentobarbital (Abbott Laboratories, Abbott Park, Illinois U.S.A.), 30 mg/Kg body weight, via the circumflex ear vein. A polyethylene catheter was inserted into the right carotid artery through which Leptin was injected and the measurement of blood pressure and heart rate were recorded by pressure transducer (Washington PT400). The MABP was calculated by this equation: $MABP = 1/3$ of systolic BP + $2/3$ diastolic BP. Another catheter was inserted into the right femoral vein for Leptin infusion in B group and to obtain blood samples for pH testing. To maintain eucapnia, trachea was cannulated and artificial respiration was instituted during the whole experiment on the rabbits. At the start of each experiment after the surgery was performed and the tubes were placed, blood pressure and heart rate were recorded via the catheter that has been inserted into the right carotid artery using Harvard Universal Oscillograph with ECG.

In the first group (Group A) Leptin hormone was infused in one shot via the carotid artery in different concentrations as follows. Subgroup A1 received 3 μ g/Kg, subgroup A2 received 5 μ g/Kg and subgroup A3 received 7 μ g/Kg of Leptin. In the second group (group B), Leptin hormone was infused via the femoral vein in the following concentration 3, 5 and 7 μ g/Kg to B1, B2 and B3 subgroups, respectively. Leptin was obtained from Diagnostic System Laboratories INC. Webster/NY, U.S.A. After the administration of Leptin to both groups, blood pressure and heart rate were measured at different time points 10, 20 and 30 minutes. The pH was monitored during all experiments to make sure that anesthetized rabbits have good ventilation. In statistical analysis, independent t-test and ANOVA tests were used. A value of $P \leq 0.05$ was considered statistically significant.

RESULTS

Table 1 demonstrates the response of mean arterial blood pressure (MABP) to Leptin infusion and summarizes the baseline values for MABP before and after intra-arterial and

intra-venous infusion of different doses of Leptin. A significant increase in MABP was seen after the intra-arterial injection of 3, 5 and 7 μ g/kg of Leptin. This increase in MABP was seen 10 minutes after the infusion of each Leptin concentrations and persisted after 20 and 30 minutes after the infusion of Leptin. The trend of the increase in MABP with time was demonstrated with all three concentrations. As shown in Table 1, we started to see the Leptin effect as early as 10 minutes after the administration of all three concentrations. The average of the increase in MABP at the end of the first 10 minutes with these concentrations compared with baseline BP was about 5.3 ± 0.6 mm Hg. This increase was also demonstrated at the end of 20 and 30 minutes after infusion, the average of the increase was 6.7 ± 1.5 and 9.3 ± 0.6 mm Hg respectively compared with controls. The differences in the average increment between 10 and 20min and between 20 and 30 min were 1.3 ± 1.2 and 2.3 ± 1.5 mm Hg respectively. As shown in Table 1, the most increase in MABP takes place during the first 10 minutes after the drug infusion. If we compare the MABP 30 minutes after infusion with baseline blood pressure for all three concentrations we will find that the increase is statistically significant. This increase was from 65 ± 4 to 75 ± 8 mm Hg (P -value = 0.003), 66 ± 7 to 75 ± 8 mm Hg (P -value = 0.001) and from 66 ± 10 to 75 ± 9 mm Hg (P -value = 0.001) for the concentrations of 3, 5, and 7 μ g/kg respectively. It is also shown in Table 1 that intra-venous infusion of Leptin resulted in a significant decrease in MABP after 10 minutes as well as after 20 and 30 minutes at all three concentrations (3, 5, and 7 μ g/Kg), with P -values equal to 0.002, 0.002 and 0.011 respectively. The obvious decrease in MABP was noted at the end of the first 10 minutes followed by a trend of decrement in the rest of the experimental time, up to 30 minutes.

The response of the heart rate (HR) to Leptin infusion is demonstrated in Table 2. Results were taken at the end of 30 minutes after intra-venous and intra-arterial Leptin infusion in three concentrations 3, 5, and 7 μ g/Kg. As shown in the table, the HR was increased at the end of 30 min of infusion but this increment was not statistically significant compared with the base line values.

Table 1. The Response of the Mean Arterial Blood Pressure (MABP) in (mmhg) on Intra-Arterial and Intra-Venous Infusion of Leptin at Different Concentrations (3, 5, and 7 μ g/Kg) Compared with Baseline Readings. P Value is Different Between Baseline Reading and Last Reading

Leptin Concentrations	Baseline BP Mmhg	BP After 10 min	BP After 20 min	BP After 30 min	P-Value
Intra-arterial Infusion (group A)					
3 μ g/Kg	65 \pm 4	71 \pm 6	73 \pm 6	75 \pm 8	0.003
5 μ g/Kg	66 \pm 7	71 \pm 6	72 \pm 6	75 \pm 8	0.001
7 μ g/Kg	66 \pm 10	72 \pm 10	74 \pm 9	75 \pm 9	0.001
Intra-venous Infusion (group B)					
3 μ g/Kg	74 \pm 10	70 \pm 11	66 \pm 9	64 \pm 7	0.002
5 μ g/Kg	80 \pm 5	72 \pm 8	69 \pm 6	68 \pm 8	0.002
7 μ g/Kg	78 \pm 9	74 \pm 8	69 \pm 8	68 \pm 8	0.011

Table 2. The Response of the Heart Rate (HR) on Intra-Arterial and Intra-Venous Infusion of Leptin at Different Concentrations (3, 5, and 7 µg/Kg) Compared with Baseline Readings

Leptin Concentration	Baseline Heart Rate beats/Minute	Heart Rate Beats/Minute After 30 min	P-Value
Intra-arterial infusion (group A)			
3µg/Kg	273±40	283±28	0.44
5µg/Kg	273±35	272±35	0.74
7µg/Kg	266±42	274±36	0.26
Intra-venous infusion (group B)			
3µg/Kg	268±27	266±26	0.42
5µg/Kg	274±30	288±31	0.07
7µg/Kg	266±38	273±36	0.35

DISCUSSION

Variable effects of Leptin on cardiovascular system were found in different studies. In the present study we demonstrated considerably new findings concerning the effect of Leptin on MABP. Intra-venous infusion of Leptin in anesthetized rabbits showed a significant decrease in MABP. This decrease in MABP could be due to the direct effect of Leptin on vascular endothelium that resulted in endothelium-dependent relaxation of small veins through the release of NO. This relaxation effect might increase the vascular capacitance and retained more blood in the small veins. The possible consequence of this effect is the reduction in cardiac filling followed by a decrease in cardiac output as well as blood pressure. This type of mechanism was demonstrated in a study performed by Hainsworth *et al.* [20]. Our findings are different from those previously obtained by other researchers. The changes in MABP that we demonstrated in the result of intra-venous Leptin administration to anesthetized rabbits were not developed in conscious rats and rabbits as previously declared. It was reported that intra-venous Leptin infusion in both conscious rabbits and rats did not affect blood pressure [21,22]. This discrepancy could be explained by more than one factor; the effect of anesthesia, the animal type, the status of the nervous system (animals being conscious or anesthetized) and the effect of Leptin effect whether on the sympathetic nervous system by increasing sympathetic activity or the direct effect on the vascular endothelium by increasing the synthesis of NO.

The significant increase in MABP after intra-arterial Leptin infusion that we were demonstrated in this study might be due to a central stimulation of sympathetic activity which caused vasoconstriction and consequently blood pressure elevation. These findings are consistent with what have been reported in the previous studies. It has been found that Leptin administered intra-cerebroventricularly or intra-venously increases sympathetic nervous activity [8,11,12]. On the other hand our results, regarding the increase in MABP after intra-arterial Leptin infusion, are inconsistent with a reasonable number of studies where implicated Leptin as vasodilator of peripheral vessels *in vitro* [9,14,22-24]. The direct vasodilator response to Leptin contrast with vasoconstrictor responses resulting from increases in sympathetic

efferent discharge which have been demonstrated when Leptin infused into both animal [8,12] and human [25].

It is also demonstrated by Knudson *et al.* that in anesthetized dogs intra-coronary Leptin infusion did not change coronary blood flow significantly [26]. They also reported that Leptin causes marked dilator response in isolated coronary arterioles. While other study on coronary blood vessels in human [27] reported that Leptin induces a dose-dependent, NO independent increase in both coronary artery diameter and coronary blood flow.

In this study intra-arterial injection of Leptin causes increases in MABP obtained at 10, 20, 30 minutes after infusion which is consistent with other study by Matsumura *et al.* [21]. This experiment also demonstrates that intra-venous or intra-arterial infusion of Leptin does not have significant effects on the heart rate in anesthetize rabbits. Possible explanations for this finding are; intra-venous infusion mostly cause direct effect on the blood vessels and induce relaxation, while intra-arterial infusion cause sympathetic stimulation but not always increase significantly heart rate in anesthetized animal. This finding also reported by other study, in rabbits with intra-cerebroventricular injection of Leptin elicited dose related increases of MABP but no consistent significant increase of heart rate [21]. Since the heart rate does not changed significantly with the changes of blood pressure after Leptin infusion by both ways, this raises the possibility the leptin is having other modulating effects on heart rate. Further studies are needed to see whether increased MABP after intra-arterial Leptin infusion is due to increased peripheral resistance or other causes.

In conclusion this *in vivo* study demonstrated that Leptin exerts direct vasodilatory action by intra-venous infusion that lead to decrease MABP. While intra-arterial infusion increases peripheral blood vessels resistance and MABP through centrally acting sympathetic stimulation. But both intra-venous and intra-arterial infusion of Leptin did not significantly change the heart rate.

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REFERENCES

- [1] Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman J. Positional cloning of the mouse obese gene and its human homologue. *Nature* 1994; 372: 425-32.
- [2] Bray GA. Leptin and leptinomania. *Lancet* 1996; 348: 140-41.
- [3] Sørensen TI, Echwald SM, Holm JC. Leptin in obesity. *BMJ* 1996; 313: 953-54.
- [4] Rohner-Jeanrenaud F, Jeanrenaud B. Obesity, leptin, and the brain. *N Engl J Med* 1996; 334: 324-5.
- [5] Campfield LA, Smith FJ, Guisez Y, Devos R, Burn P. Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. *Science* 1995; 269: 546-49.
- [6] Halaas JL, Gajiwala KS, Maffei M, *et al.* Weight-reducing effects of the plasma protein encoded by the obese gene. *Science* 1995; 269: 543-46.
- [7] Pelleymounter MA, Cullen MJ, Baker MB, *et al.* Effects of the obese gene product on body weight regulation in ob/ob mice. *Science* 1995; 269: 540-43.
- [8] Dunbar JC, Hu Y, Lu H. Intra-cerebroventricular leptin increases lumbar sympathetic and renal sympathetic nerve activity and blood pressure in normal rats. *Diabetes* 1997; 46: 2040-43.
- [9] Lembo G, Vecchione C, Fratta L, *et al.* Leptin induces direct vasodilatation through distinct endothelial mechanism. *Diabetes* 2000; 49: 293-97.
- [10] Julius S, Valentini M, Palatini P. Overweight and hypertension a 2-way street? *Hypertension* 2000; 35: 807-13.
- [11] Haynes WG, Sivitz WI, Morgn DA, Walsh SA, Mark AL. Sympathetic and cardiorenal actions of leptin. *Hypertension* 1997; 30: 619-23.
- [12] Rahmouni K, Haynes WG, Mark AL. Cardiovascular and sympathetic effects of leptin. *Curr Hypertens Rep* 2002; 4: 119-25.
- [13] Bouloumie A, Drexler HCA, Lafontan M, Busse R. Leptin, the product of Ob gene, promotes angiogenesis. *Circ Res* 1998; 83: 1059-66.
- [14] Kimura K, Tsuda K, Baba A, *et al.* Involvement of nitric oxide in endothelium-dependent arterial relaxation by leptin. *Biochem Biophys Res Commun* 2000; 273: 745-49.
- [15] Vecchione C, Maffei A, Colella S, *et al.* Leptin effect on endothelial nitric oxide is mediated through Akt-endothelial nitric oxide synthase phosphorylation pathway. *Diabetes* 2002; 51: 168-73.
- [16] Mohammed MJ, David SM, Lucy JH, Roger H, Mark JD. Effects of dietary salt loading on the responses of isolated rat mesenteric arteries to leptin. *Am J Hypertens* 2005; 18(4): 500-03.
- [17] Agata J, Masuda A, Takada M, *et al.* High plasma immunoreactive leptin level in essential hypertension. *Am J Hypertens* 1997; 10: 1171-74.
- [18] Soderberg S, Ahren B, Jansson JH, *et al.* Leptin is associated with increased risk of myocardial infarction. *J Intern Med* 1999; 246(4): 409-18.
- [19] Shek EW, Brands MW, Hall JE. Chronic leptin infusion increases arterial pressure. *Hypertension* 1998; 31: 409-14.
- [20] Hainsworth R. Vascular capacitance its control and importance. *Rev Physiol Biochem Pharmacol* 1986; 105: 101-03.
- [21] Jennifer LM, Donald AM, Marcelo LC, Allyn LM, William IS, William GH. Does leptin stimulate nitric oxide to oppose the effects of sympathetic activation. *Hypertension* 2001; 38: 1081-86.
- [22] Matsumura K, Abe I, Tsuchihashi T, Fujishima M. Central effects of leptin on cardiovascular and neurohormonal responses in conscious rabbits. *Am J Physiol* 2000; 278: 1314-20.
- [23] Nakagawa K, Higashi Y, Sasaki S, Oshima T, Matsuura H, Chayama K. Leptin causes vasodilation in human. *Hypertens Res* 2002; 25: 161-65.
- [24] Winters B, Mo Z, Brooks-Asplund E, *et al.* Reduction of obesity, as induce by leptin, reverses endothelial dysfunction in obese (Lep ob) mice. *J Appl Physiol* 2000; 89: 2382-90.
- [25] Haynes WG, Morgan DA, Wash AL, Sivitz WI. Leptin increases sympathetic nerve activity to brown adipose tissue and kidney. *FASEB J* 1997; 11. (Abstract) A4.
- [26] Knudson JD, Dincer UD, Zhang C, *et al.* Leptin receptors are expressed in coronary arteries, and hyperleptinemia causes significant coronary endothelial dysfunction. *AJP, Heart Circ Physiol* 2005; 289: 48-56.
- [27] Matsuda K, Teragawa R, Fukuda Y, Nakagawa K, Higashi Y, Chayama K. Leptin causes nitric-oxide independent coronary vasodilatation in human. *Hypertens Res* 2003; 26: 147-52.

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