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*Original Contribution*

# ENHANCED FOOD INTAKE SUPPRESSION AND BODY MASS REDUCTION WITH COMBINED L-NAME AND LEPTIN ADMINISTRATION IN FASTED RATS

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**ABSTRACT**
**PURPOSE**: This study aimed to investigate the effect of combining Nω-Nitro-L-arginine methyl ester (L-NAME) and leptin on food intake and body mass gain in fasted rats, as both have been shown to suppress food consumption. Additionally, the study evaluated the impact of L-NAME on fever induced by leptin.
**METHODS:** L-NAME and recombinant rat leptin were administered intraperitoneally, both alone and in combination, to fasted rats. Measurements included monitoring body temperature via rectal thermocouple probes, and assessing food intake and body mass gain 24 hours post-injection.
**RESULTS**: Systemic administration of L-NAME (50 mg/kg, i.p.) abolished the febrile response elicited by leptin. Furthermore, administering leptin (0.5 mg/kg, i.p.) and L-NAME (50 mg/kg, i.p.), alone or in combination, suppressed food intake and body mass gain in fasted rats after a 24-hour period. Notably, combining leptin and L-NAME had a more pronounced effect on food intake suppression and body mass loss than using either drug alone.
**CONCLUSIONS**: It could be speculated that the combination of leptin and L-NAME might potentially serve as an effective treatment for obesity, given their enhanced effects on food intake suppression and body mass loss observed in the study. Additionally, these results suggest a clear involvement of nitric oxide synthase during leptin-induced fever.

**Key words:** nitric oxide, L-NAME, leptin, food intake, body mass, body temperature, fever

## INTRODUCTION

Nitric oxide (NO) is a gasotransmitter, synthesized by three isoforms of nitric oxide synthase (NOS): neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS) (1). A growing body of literature suggests that NO plays a crucial role in regulating both food intake and body mass. For example, various feeding-related peptides such as ghrelin, neuropeptide Y, orexin-A, and cholecystokinin exert their effects through the activation of NOS (2). It has been demonstrated that eNOS-deficient mice display a lower metabolic rate and faster body mass gain compared to wild-type mice (3). Another study has reported that knocking down iNOS in the dorsal vagal complex of obese rats leads to reduced food intake, lower body mass gain, and decreased adipose tissue (4). Research indicates that NO also influences body temperature control (1). It has been shown that when iNOS or nNOS is deficient, lipopolysaccharide-induced fever is affected, while eNOS deficiency affects turpentine-induced fever (5).
Leptin is an adipokine that regulates energy balance by decreasing appetite and increasing thermogenesis in brown adipose tissue. Upon binding to the functional form of its receptor in the hypothalamus, leptin activates neurons expressing anorexigenic peptides and inhibits neurons expressing orexigenic peptides (6, 7). Leptin has been demonstrated to induce a fever, equivalent in magnitude to that elicited by lipopolysaccharide or cytokines, such as interleukin-1 beta (8, 9). Numerous studies suggest that NO plays a vital role in mediating leptin's effects (10-13).

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Nω-Nitro-L-arginine methyl ester (L-NAME) is widely used as a potent competitive inhibitor of all isoforms of NOS (a nonselective NOS inhibitor) (14). Previous studies using L-NAME have demonstrated its ability to reduce food intake and body mass gain in both mice and rats (15-17). The purpose of this study was to investigate the effects of combining leptin and L-NAME on food consumption and body mass gain in fasted rats, as both have been shown to suppress food intake. The study also examined the impact of L-NAME on leptin-induced fever.

## MATERIALS AND METHODS

### Drugs

Nω-Nitro-L-arginine methyl ester (N5751) and recombinant rat leptin (L5037) were purchased from Sigma-Aldrich, Schnelldorf, Germany. L-NAME was injected at a dose of 50 mg/kg (17), and leptin was injected at a dose of 0.5 mg/kg (7, 18). The drugs were dissolved in saline (0.9% w/v NaCl) and administered intraperitoneally (i.p.) at an injection volume of 0.2 ml/100 g of body mass. The control animals received saline (0.9% w/v NaCl).

### Animals

Male Wistar rats aged 10-12 weeks with a body mass of 275±25 g were procured from the Laboratory Animal Breeding Center of the Bulgarian Academy of Sciences located in Slivnitsa, Bulgaria. They were group-housed in sets of three per cage in a temperature-regulated environment (20-22 °C) on a 12:12-hour light-dark cycle (07:00 to 19:00 h). These rats were provided unrestricted access to standard chow pellets (HL-TopMix, Sliven, Bulgaria) and water. All animal experiments were conducted in accordance with the guidelines outlined in Directive 2010/63/EU of the European Parliament and of the Council, dated 22 September 2010, pertaining to the protection of animals utilized for scientific purposes, and to the 'Guide to the Care and Use of Experimental Animals' (Canadian Council on Animal Care guidelines, 1984). The Ethical Council of the Bulgarian Food Safety Agency (Approval Number: 315) granted consent for all experimental procedures.

### Measurement of food intake, body mass, and body temperature

The experimental protocol employed in this study closely followed a previously established method (18). Before the experiment, all rats underwent a 24-hour food deprivation period (water access was maintained). The rats' body masses were measured both prior to and 24 hours following the injections. To ensure consistent conditions, all experimental procedures were conducted between 10:30 AM and 11:30 AM to mitigate potential effects linked to circadian rhythms. Rats were intraperitoneally injected first with L-NAME or saline, followed by a subsequent injection of leptin or saline after a 10-minute interval. The animals were categorized into four treatment groups, each consisting of six animals: saline+saline, saline+leptin, L-NAME+saline, and L-NAME+leptin. Body temperature was meticulously monitored utilizing thermocouple probes, which were connected to a computer-controlled multi-channel thermocouple thermometer Iso-Thermex (Columbus Instruments, Columbus, Ohio, U.S.A.). Thermocouple probes were lubricated with petroleum jelly and inserted at least 6 cm into the rectum to accurately track core body temperature. The rats' movements were mildly restricted for the temperature monitoring duration. Initial body temperature readings were taken right before administering the first injection, revealing a consistent range of 37.2 to 37.8 °C across all rats. Post the second injection, body temperature was recorded at 30-minute intervals over 150 minutes. To assess the overall change in body temperature from -10 to 150 minutes post-injection, the area under the curve (AUC) was computed. This was accomplished using the built-in "Area Below Curves" macro in SigmaPlot 12.5 (Systat Software GmbH, Erkrath, Germany). Each animal was utilized for the experiments only once. After the body temperature measurements, the rats were placed in separate cages within 5 minutes. Pre-weighed chow pellets were provided in each cage, and food intake measurements were gathered 24 hours post-injection. Food weight was adjusted to account for spillage during measurement. The entire experimental setup was maintained at a constant room temperature ranging from 20°C to 22°C.

### Statistical analysis

Statistical analysis was conducted using SigmaPlot 12.5 software (Systat Software GmbH, Erkrath, Germany). Normality testing was performed using the Shapiro-Wilk test, confirming that the data exhibited a normal distribution. Data originating from two treatment groups (ΔTemperature) were analyzed using a two-tailed Student's t-test. For comparisons among more than two treatment groups (The AUC, food intake, and body mass change), a one-way ANOVA was employed, followed by the Student-Newman-Keuls multiple comparison test. Statistical significance was set at a p-value less than 0.05. All values are presented as the mean ± standard error of the mean (SEM).

## RESULTS

### Effects of L-NAME and leptin, administered alone and in combination, on food intake and body mass gain in fasted rats

Peripheral administration of L-NAME (50 mg/kg, i.p.) and leptin (0.5 mg/kg; i.p.), either separately or in combination, had a significant impact on both food consumption **(Figure 1a**, $F_{3,20}= 28.706$, $p < 0.001$, power of performed test with alpha = 0.050 is 1.000) and the change in body mass **(Figure 1b**, $F_{3,20}= 41.934$, $p < 0.001$, power of performed test with alpha = 0.050 is 1.000) in rats that had undergone fasting. Further analysis indicated that animals administered with L-NAME, leptin, or a combination of both, displayed reduced food intake (Fig. 1a) and a decrease in body mass gain **(Figure 1b)** compared to the control group. The effects on food consumption and body mass gain noted following the administration of L-NAME were not significantly different from the results observed after the administration of leptin (L-NAME/saline vs. saline/leptin: $p > 0.068$, **Figure 1a, b**). Interestingly, the response of suppressed food intake and body mass loss induced by the combined administration of L-NAME and leptin showed a statistically significant difference compared to the responses observed in animals treated with leptin or L-NAME.

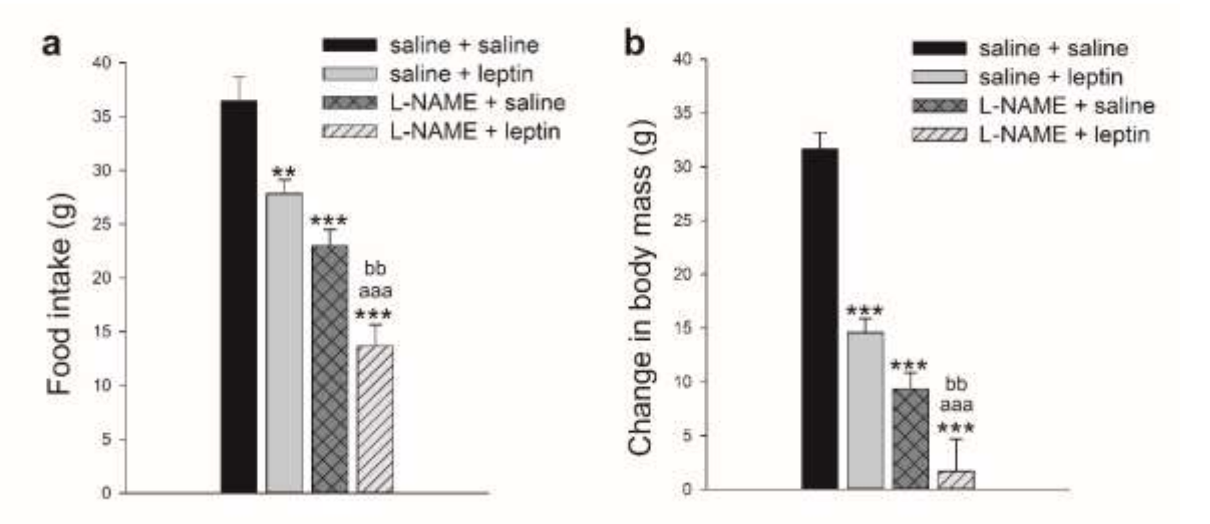


**Figure 1.** Effects of L-NAME and leptin, administered alone and in combination, on food intake (a) and body mass gain (b) in 24-hour fasted rats. Systemic administration of L-NAME, leptin, or a combination of both, induced a reduction in food intake and body mass gain at 24 hours post-injection. The results are presented as the mean ± SEM. n=6 rats per group. **, p< 0.01; ***, p < 0.001 vs. saline+saline group; $^{aaa}$, p < 0.001 vs. saline+leptin group; $^{bb}$, p < 0.01 vs. l-NAME+saline group.

### Effects of L-NAME and leptin, administered alone and in combination, on core body temperature in fasted rats

As depicted in **Figure 2a**, the administration of L-NAME (50 mg/kg, i.p.) did not significantly affect core body temperature when compared to the rats treated with saline (all $p > 0.17$). Throughout the 150-minute recording period, the combined administration of L-NAME (50 mg/kg, i.p.) and leptin (0.5 mg/kg, i.p.) entirely abolished the leptin-induced febrile response **(Figure 2b).** Analysis of the AUC indicated that the differences in mean values among the treatment groups were greater than would be expected by chance **(Figure 3**, $F_{3,20}= 6.909$, $p = 0.002$, power of performed test with alpha = 0.050 is 0.915). A post hoc test conducted on the mean AUC values revealed a significant difference in the L-NAME/leptin group compared to the saline/leptin group. Also, no significant differences were observed when comparing the L-NAME/leptin group to the saline/saline group or the L-NAME/saline group **(Figure 3).**

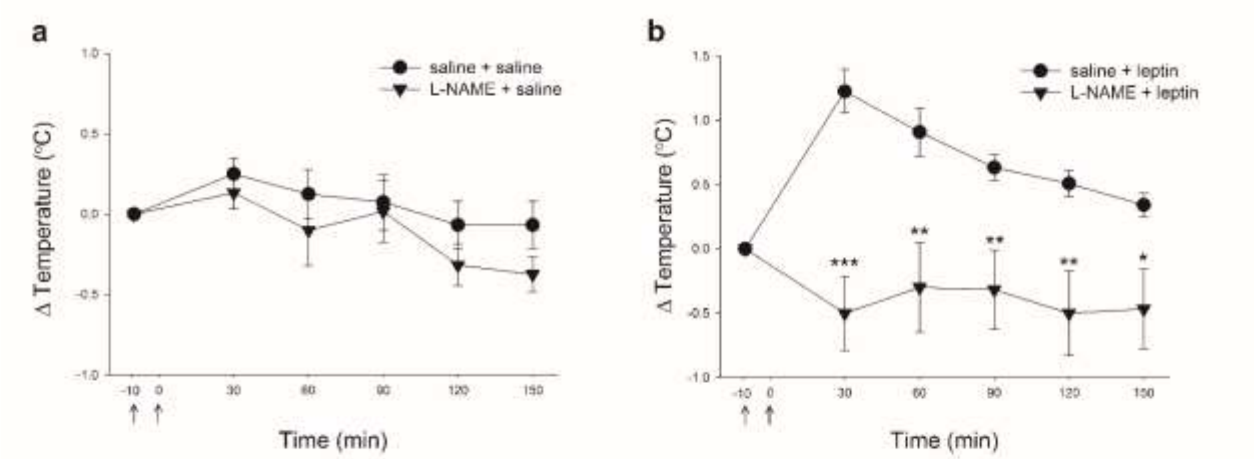


**Figure 2.** Effects of L-NAME and leptin, administered alone and in combination, on core body temperature in 24-hour fasted rats. The first arrow indicates the time point of the initial injection (either L-NAME or saline), while the second arrow indicates the time point of the subsequent injection (either saline or leptin). In panel (a), intraperitoneal injection of L-NAME did not induce a change in body temperature. In panel (b), the combined administration of L-NAME with leptin completely abolished the leptin-induced febrile response. ΔTemperature represents the change in body temperature from the baseline (time -10). All results are presented as the mean ± SEM. n=6 rats per group. *, p< 0.05; **, p< 0.01; ***, p < 0.001 vs. saline+leptin group.

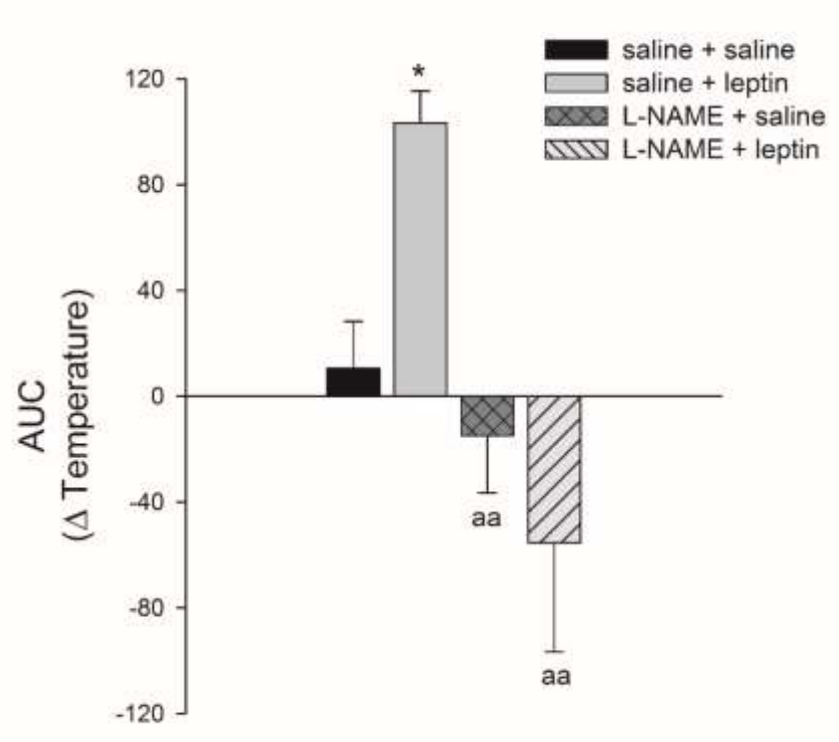


**Figure 3.** The area under the curve (AUC) was calculated to assess the overall change in body temperature from -10 to 150 minutes post-injection. The results are presented as the mean ± SEM. n=6 rats per group. *, p< 0.05 vs. saline+saline group; $^{aa}$, p < 0.01 vs. saline+leptin group.

## DISCUSSION

In this study, it was found that administering leptin and L-NAME, alone or in combination, suppressed food intake and body mass gain in fasted rats after a 24-hour period. Combining leptin and L-NAME resulted in greater effects than using the drugs alone. We have previously shown that administering leptin with the selective nNOS inhibitor 7-nitroindazole or the selective iNOS inhibitor aminoguanidine produces similar effects as the drugs alone (18). A possible reason for the difference in results may be due to the complex mechanism of the anorexigenic action of L-NAME, which may include an effect on gastric accommodation. Prior research has shown that all NOS isoforms are expressed in smooth muscle cells and myenteric neurons of the gastrointestinal tract (19). Systemic administration of L-NAME resulted in a higher intragastric pressure increase during nutrient infusion in rats compared to controls, suggesting that gastric accommodation is meditated by NO (20). It has been demonstrated that L-NAME inhibits the electrically induced relaxations in the porcine gastric fundus, whereas 7-nitroindazole and aminoguanidine do not (21). Additionally, nonselective inhibition of NOS in humans has been reported to inhibit gastric accommodation to a meal accompanied by increased satiety scores during meal intake and a significant reduction in the amount of calories ingested at maximum satiety (22).

The inability of leptin to suppress appetite and decrease body mass, known as leptin resistance, is a common feature of diet-induced obesity (7, 11). Although leptin-based therapy has been effective in promoting body mass loss in genetically predisposed obese individuals with leptin gene mutations, it has limited or no effect on body mass loss in individuals with common obesity, particularly those with high leptin levels (23). The mechanisms underlying leptin resistance appear to cause a global impairment of satiety-related vagal afferent responsiveness. A recent study has found that inhibition of iNOS during obesity can enhance satiety signalling associated with leptin action (24). Another study has reported that diet-induced obese rats are more sensitive to the effects of L-NAME on food intake and body mass gain than lean controls (16). Therefore, it could be hypothesized that administering leptin and L-NAME together could effectively induce appetite suppression and body mass loss in obesity. The direction for future research should prioritize the exploration of novel mechanisms governing leptin regulation at the whole-body level. This will facilitate the development of therapeutic agents aimed at mitigating leptin resistance (23).

In the present study, it was also found that inhibiting NO synthesis by systemic administration of L-NAME abolished the febrile response elicited by leptin, suggesting a clear involvement of NOS during leptin-induced fever. These results are consistent with a body of literature that supports the concept of NOS as a mediator of fever and different forms of hyperthermia (1, 18, 25, and 26). Previous studies have shown that obese leptin receptor-deficient (fa/fa) Zucker rats have various thermoregulatory deficits, such as lower body temperature and reduced thermogenic responses to stress, cold exposure, and fever (27-29). Lean Zucker rats experienced a significant drop in body temperature after administration of L-NAME or 7-nitroindazole, whereas obese (fa/fa) Zucker rats were unaffected (29). Furthermore, L-NAME and 7-nitroindazole attenuated hypoxia-induced hypothermia or hypometabolism in lean Zucker rats. However, in obese (fa/fa) Zucker rats, there was no such effect, suggesting that reduced activity of NOS in the central nervous system may cause the blunted thermoregulatory response to hypoxia in these rats (29). Therefore, it could be suggested that leptin-induced nitric oxide production in the central nervous system may be required for intact thermoregulation.

## CONCLUSION

In this study, it was demonstrated that inhibiting NO synthesis using L-NAME abolished the febrile response caused by leptin. Additionally, administering either leptin or L-NAME, or both, reduced food intake and body mass gain in fasted rats after 24 hours. Combining leptin and L-NAME had a greater effect on food intake suppression and body mass loss than using either drug alone. It could be speculated that the combination might potentially serve as an effective treatment for obesity.

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**Conflict of interests:** The author has no competing interests to disclose.

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