

role of leptin and tumor necrosis factor- α (tnf- α) in mechanisms of anorexia during endotoxin infection in mice

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ABSTRACT

Background: Anorexia and loss of body weight are hallmark of infection. The actual mechanisms by which infection induces anorexia are still unknown. Several pro-inflammatory cytokines, most notably tumor necrotic factor- α (TNF- α), known to initiate and modulate the host response to infection have been shown to induce anorexia and weight loss in healthy animals. Leptin is a protein hormone produced by adipose tissue. It is considered to be a satiety factor as it decreases food intake and increases energy expenditure. Administration of bacterial endotoxin (LPS) or TNF- α induced increase in serum leptin levels, and leptin has been shown to act in an endocrine fashion to decrease food intake and body weight. This suggests that leptin may be one of the mechanisms by which anorexia is induced during infection. The present study aimed to investigate the effect of endotoxin (LPS) and TNF- α , on food intake, body weight and serum leptin levels in mice. Also, to explain the different possible mechanisms which can cause anorexia during infection and if leptin is involved in these mechanisms. **Methods:** The study included 36 adult female mice which were divided into 6 groups, 6 mice each. Group I: "control group", received single intraperitoneal (i.p) injection of normal saline. Group II, III, IV and V: "LPS treated groups", received a single i.p. injection of different doses of LPS, (0.1 μ g, 1 μ g, 10 μ g and 100 μ g/100g. body weight respectively). Group VI: "TNF- α treated group", injected i.p. with TNF- α in a dose of 17 μ g/100g BW. Food intake and body weight were measured over the next 18, 42, 66 and 90 h for animals of control and LPS treated groups. Food intake by TNF- α injected group was measured 18 h after injection. Blood sample from each mouse of all studied animal groups was collected 18 h after different injections, then serum leptin level was assessed using mouse leptin ELISA kits. **Results:** The study showed a significant (p is < 0.001), dose dependent decrease in food intake and body weight 18 h after injection in all LPS injected groups (except for group II which showed a non significant decrease) when compared with that of control group. Gradual recovery of food intake and body weight gaining over the next 3 days occurred in low doses treated groups (groups II & III), while as mice treated with large doses, (group VI & V), showed no food intake nor weight gain over the subsequent 42 h. Thereafter, group IV began to increase their food intake and body weight till the end of the study, whereas group V remained anorectic and losing weight till the end of the study. TNF- α injected group showed a significant decrease in food intake, (p is < 0.001) as compared to the control one, that decrease in food intake after TNF- α injection is nearly similar to that induced by LPS

injection even in high doses injected groups (groups III & IV). Serum leptin levels showed significant increase 18 h after LPS and TNF- α injection in all injected groups in a dose related manner, compared to that of the control group. The highest dose of LPS injection to group V "100 μ g / 100 g BW" showed increased serum leptin levels nearly 4 folds to that level of control group. The increase in serum leptin levels after TNF- α injection, did not reach the same levels of increase as after LPS injections specially for the high doses of LPS "groups IV and V". **Conclusion:** The present study showed that both of LPS and TNF- α can induce anorexia and increased leptin level. The decrease in food intake is inversely proportional to the increase in serum leptin. These data suggest a role for leptin in anorexia during LPS infection. Also, the study revealed some possible mechanisms for anorexia during infection through the cooperation between immune activation mediators (cytokines) and some hormonal changes which can induce different host's immune and metabolic response during infection. As anorexia plays a critical role in chronic inflammatory diseases, it is possible that development of leptin antagonists may play a useful role in decreasing anorexia and wasting of chronic infections such as AIDS.

INTRODUCTION

Anorexia and loss of body weight are important consequences of infection^(1,2). Although anorexia is a common behavioral response to infectious diseases, the reason for and mechanisms behind that observation are still unknown⁽³⁾. The first response to infection is the development of acute phase response (APR) which is characterized by production of several cytokines known to induce anorexia. Several microbial products and cytokines reduce food intake after parenteral administration suggesting a role of these substances in anorexia during infection⁽³⁾.

Several inflammatory cytokines, most notably tumor necrosis factor- α (TNF- α) and interleukin-1 (IL-1), induce anorexia and loss of body weight in healthy animals. Administration of TNF- α or IL-1 produces a prompt and dose-dependant increase in serum leptin levels, and leptin has been shown to

act in an endocrine fashion to decrease food intake and body weight⁽⁴⁾.

Leptin is a 16 kDa protein mainly produced by adipose tissue in proportion to adipose tissue mass^(5,6). Later works have revealed that placental tissue⁽⁷⁾, myocytes⁽⁸⁾ and fat cells derived from bone marrow cell cultures⁽⁹⁾ can express leptin mRNA.

The word Leptin is coming from the Greek *Leptos*, which means thin. It was originally identified as the gene defect responsible for the obesity syndrome in *ob/ob* mice in 1994 by Friedman⁽¹⁰⁾ and colleagues, which were characterized by severe obesity due to both overeating and decreased energy expenditure. The gene was named *ob* and the obese mice carrying the mutation were called *ob/ob* mice. Leptin is the protein encoded by the *ob* gene and it is considered to be a satiety factor as it decreases food intake and increases energy expenditure.

Recent studies indicate that food intake and body weight are regulated by leptin. Infusion of leptin decreases food intake and body weight in both

obese and normal mice^(11,12). Intracerebral infusion of leptin is even more effective than peripheral infusion⁽¹²⁾. Infusion of leptin decreases hypothalamic levels of neuropeptide Y (NPY) which is known as a potent orexigenic peptide⁽¹³⁾.

Many cytokines and bacterial endotoxins as LPS (bacterial cell wall product lipopolysaccharide) that mediate the host response to infection are well known to regulate adipose tissue metabolism⁽¹⁴⁾. Several hormones known to regulate appetite, including corticotropin releasing hormone, cholecystokinin, prostaglandins, glucagon and insulin are induced by LPS and cytokines^(15,16,17). Also, it is known that leptin secretion can be regulated by inflammatory markers such as IL-1 and TNF- α ⁽¹⁸⁾. Therefore, it might be postulated that LPS and, or cytokines as TNF- α could induce leptin production in adipose tissue during infection. Increased circulating leptin levels during infection could decrease food intake suggesting that leptin may contribute to the anorexia of infection^(19,20).

Aim of the work:

The aim of the present work was to investigate the effect of injected endotoxin (LPS), a model of gram negative bacterial infection, and the pro-inflammatory cytokine, TNF- α , on food intake, body weight and serum leptin levels in mice. Also, to explain the different possible mechanisms which can cause anorexia during infection and if leptin induction by TNF- α can play a role in anorexia during infection in mice.

MATERIALS & METHODS

(A) Animals and Reagents:

1. Experimental animals:

The study was conducted on adult female mice. Animals were obtained from the Animal House of Faculty of Medicine, Assiut University. Their weight ranged 19-20 g. at the beginning of the experiment. They were housed in groups (6 / cage) in standard environmental condition including good aerated room with suitable temperature. All animals had free access to water and standard rodent chow. The animals were adapted to the cages for several days before the beginning of the experiment. The study included 36 adult female mice which were divided into 6 groups, 6 mice each.

2. Reagents:

a. Endotoxine: LPS "bacterial cell wall product lipopolysaccharide from Echerichia Coli Serotype 0128:B8 from Sigma Chemical Co. (st. Louis. Mo). It was dissolved in sterile phosphate buffered saline (PBS) (0.9g% NaCl; Sigma Chemical) for i.p. "intra-peritoneal" injection.

b. Tumor Necrosis Factor- α "TNF- α " Mouse: it was obtained from KOMA Biotech Product Description, Catalog No: K0921181. Endotoxin level is less than 0.1ng/1 μ g of mouse TNF- α .

c. Mouse Leptin ELISA Kits: Leptin levels were determined using a commercial radio-immunoassay (RIA) kits which were obtained from KOMA Biotech Product Description, Catalog No: Ko331250.

(B) Methods:**1. Animals grouping:**

The animal groups were as follows:

Group I: "control group":

It included 6 mice, each mouse received a single i.p. injection of normal saline.

Group II, III, IV and V: "LPS treated groups".

Each animal of these groups received a single i.p. injection of different doses of LPS, (0.1µg, 1µg, 10µg and 100µg/100g. body weight respectively).

Group VI: "TNF-α treated group".

6 mice, each was injected i.p. with TNF-α in a dose of 17µg / 100g BW.

2. Experimental protocol:

The total time of the experiment was 90 hour for the LPS treated groups and 18 hour for TNF-α treated group. The time of starting injection is considered zero hour (0 h).

I- Study of food intake:

All animal groups (groups I to VI) were adapted to their cages and received normal standard food and water for one week before the beginning of the experiment. After i.p. injection of saline (for group I), different doses LPS (for groups II, III, IV and V) or TNF-α (for group VI), food intake for each group was determined over the next 18 h. Subsequently, food intake was monitored every 24 h for 3 more days (at 42, 66 and 90 h) for control and LPS treated groups only.

II. Study of body weight:

All mice of control and LPS treated groups (group I to group V), were weighed on the first day of the experiment before (0 h), and 18 h after

injection of LPS or saline. Subsequently, body weight of each mouse was monitored every 24h for 3 more days (at 42, 66 and 90 h).

III- Study of serum leptin levels:

Blood sample from each mouse of all studied animal groups (groups I, II, III, IV, V and VI) was collected 18 h after different injections. Blood samples were obtained from the retro-orbital plexus. Blood was centrifuged in 500 rpm for 15 minutes and serum from each sample was kept at -70° C till the time of assay.

Serum leptin levels for all treated groups of the study were assessed using a commercial RIA kits specific for mice, (Mouse Leptin ELISA Kits).

The assay procedure was carried out according to the method described in the kits catalog (Catalog No: Ko331250).

Statistical analysis:

All values were expressed as the mean ± standard error (M±SE). The statistical significance between the different groups was analyzed by student paired *t*- test. A value $p < 0.05$ was considered to represent a significant difference. Comparison of all groups was done versus control group.

RESULTS**1. Effect of LPS on food intake:**

Table (1) shows all food intake in grams by the control group and LPS treated groups before, and over the next 18 h after injection and every 24 h thereafter for 3 more days (at 42, 66 and 90 h). It showed that there was a dose dependent decrease in food intake in all LPS injected groups

(groups II, III, IV and V) as compared to control group:

Group II: (injected with the lowest dose of LPS, 0.1 µg /100g B.W) showed non significant decrease in food intake during the first 18 h when compared to that of control group ($p > 0.05$). After that, food intake returned towards normal till the end of the study.

Groups III, IV and V: (1µg, 10µg and 100µg /100gm BW respectively) showed a highly significant, dose dependent reduction in food intake over the first 18h ($p < 0.001$).

Mice treated with 1µg LPS /100g BW (group III) showed gradual recovery of food intake over the next 3 days, while mice treated with 10 or 100 µg LPS/100g BW (group VI & V), had no food intake over the subsequent 42 h. Thereafter, group IV (10µg LPS/100g BW) began to increase their food intake till the end of the study, whereas group V (100 µg LPS /100g BW) remained anorectic over the entire 90 h of the study. **Table (1) & Figure (1).**

2. Effect of LPS injection on body weight:

Table (2) shows body weight of each mouse in grams for the control group and LPS treated groups before, and over the next 18 h after injection and every 24 h thereafter for 3 more days (at 42, 66 and 90 h). Group 1 (control group) showed progressive weight gain during the experiment. Also, there was a dose dependent decrease in body weight in all LPS injected groups (groups II, III, IV and V) when compared with that of control group. Group II (treated with 0.1µg LPS/100g BW) showed statistically non significant decrease

($p > 0.05$) in their body weight 18 h after LPS injection. But, in the next 3 days, they began to gain weight.

Mice given higher doses of LPS (groups III, IV and V) showed highly significant, dose dependant progressive weight loss over the first 42 h after LPS injection ($p < 0.001$). After that, group III “1 µg/100 g BW” began to regain weight but they did not reach to the control weight while the other two groups, “IV and V” showed continuous, highly significant weight loss during the whole 90 h of the experiment. To the end of the experiment (90 h), these 3 groups showed significant decrease in their body weight as compared to control one ($p < 0.01$). **Table (2) & Figure (2).**

3. Effect of LPS injection on serum leptin levels:

Table (3) shows serum leptin levels, 18 h after injection of saline or different doses of LPS for the control group “group I” and 4 LPS injected groups “groups II, III, IV and V”.

Serum leptin levels were significantly increased 18 h after LPS injection in all injected groups in a dose related manner, compared to that of the control group ($p < 0.05$ for group II & $p < 0.001$ for the remaining groups). The highest dose of LPS injection to group V “100 µg/100g BW” showed increased serum leptin levels nearly 4 folds of that of control group, **Table (3) & Figure (3).**

4. Effect of TNF-α injection on food intake:

Figure (4) shows that 18 h after injection of group VI with 17µg / 100 g BW TNF-α, there was a highly significant decrease in food intake in that group as compared to the control group ($p < 0.001$). The degree of

decrease in food intake in TNF- α injected group is nearly similar to that induced by large doses of LPS injection, (groups III& IV).

5. Effect of TNF- α on serum leptin levels:

Figure (5) shows that there was a highly significant increase in serum

leptin levels 18 h after injection of TNF- α to group VI as compared to that of control group ($p < 0.001$). But, that increase in serum leptin levels after TNF- α injection, did not reach the same levels of increase as after injections of large doses of LPS, specially for groups IV and V.

Table (1): Effect of injection of different doses of LPS ($\mu\text{g} / 100\text{g BW}$) on total food intake (in gram) by each injected group compared to control group over different time intervals through the whole 90 h after injection

Groups Time in h (0-90 h)	Group I	LPS injected groups			
		Group II	Group III	Group IV	Group V
0-18 h P value	2.81	2.36 NS	0.82 ***	0.42 ***	0.23 ***
18-42 h P value	3.24	2.82 NS	1.61 **	0.00 ***	0.00 ***
42-66 h P value	3.31	3.28 NS	2.33 *	0.64 ***	0.00 ***
66-90 h P value	3.26	3.18 NS	3.12 NS	1.68 **	0.00 ***

N.B.: P value of all groups was versus control group. **Group I:** control group (NS): non significant at probability $p > 0.05$. **Group II:** (0.1 $\mu\text{g}/100\text{g BW}$) LPS. (*): Significant at probability $p < 0.05$. **Group III:** (1 $\mu\text{g}/100\text{g BW}$) LPS. (**): Significant at probability $p < 0.01$. **Group IV:** (10 $\mu\text{g}/100\text{g BW}$) LPS. (***) : Highly-significant at probability $p < 0.001$. **Group V:** (100 $\mu\text{g}/100\text{g BW}$) LPS.

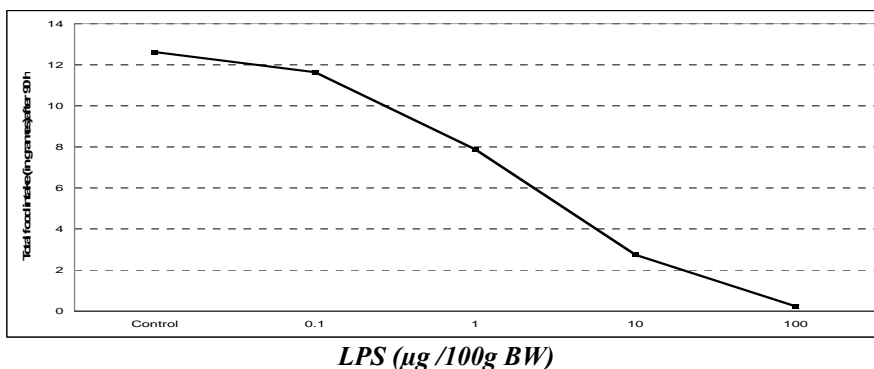


Fig.(1): Total food intake by each group (in gram) 90 h after LPS injection for all groups

Table (2): Effect of injection of different doses of LPS (μg /100g BW) on body weight (in gram) of each mouse in all LPS injected groups compared to control group over different time intervals through the whole 90 h after injection

Time in h (0-90 h)	Group I	LPS injected group			
		Group II	Group III	Group IV	Group V
0 h P value	19.64 \pm 0.13	19.67 \pm 0.13 NS	19.72 \pm 0.12 NS	19.53 \pm 0.16 NS	19.73 \pm 0.12 NS
0-18h P value	19.67 \pm 0.16	19.35 \pm 0.14 NS	17.03 \pm 0.08 ***	16.59 \pm 0.05 ***	15.98 \pm 0.04 ***
18-42 h P value	19.79 \pm 0.16	19.49 \pm 0.15 NS	16.18 \pm 0.04 ***	14.62 \pm 0.05 ***	14.08 \pm 0.05 ***
42-66 h P value	19.95 \pm 0.11	19.68 \pm 0.10 NS	16.68 \pm 0.05 ***	14.28 \pm 0.04 ***	13.67 \pm 0.05 ***
66-90 h P value	20.11 \pm 0.09	19.76 \pm 0.14 NS	17.21 \pm 0.04 **	14.16 \pm 0.05 ***	13.19 \pm 0.05 ***

Values are mean \pm SE.

N= 6 mouse/ group.

N.B.: P value of all groups was versus control group. **Group I:** control group

0 h: Time just before starting injection.

Group II: (0.1 μg /100g BW) LPS.

(NS): Non significant at probability $p > 0.05$.

Group III: (1 μg /100g BW) LPS.

():** Significant at probability $p < 0.01$.

Group IV: (10 μg /100g BW) LPS.

(*):** Highly-significant at probability $p < 0.001$. **Group V:** (100 μg /100g BW) LPS.

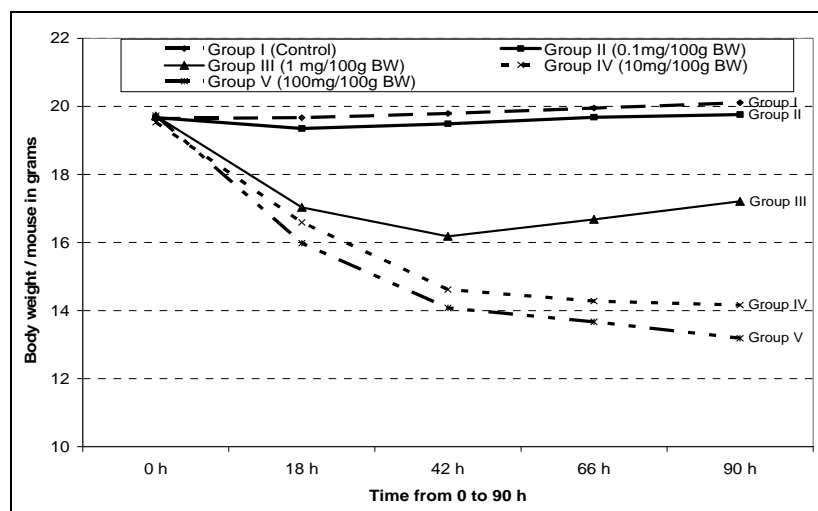


Fig. (2): Body weight changes in control & LPS groups over 90 h after LPS injection

Table (3): Effect of injection of different doses of LPS ($\mu\text{g} / 100\text{g BW}$) on serum leptin levels (ng/ml) 18 h after injection in LPS injected groups compared to control group

Groups Leptin Level ng/ml	Group I	LPS injected group			
		Group II	Group III	Group IV	Group V
Serum leptin	5.46 \pm 0.11	6.12 \pm 0.22	9.56 \pm 0.06	13.64 \pm 0.10	19.45 \pm 0.12
P value		*	***	***	***

Values are mean \pm SE.

N= 6 mouse/ group.

N.B.: P value of all groups was versus control group.

(*): Significant at probability $p < 0.05$.

(***): Highly significant at probability $p < 0.001$.

Group I: control group

Group II: (0.1 $\mu\text{g}/100\text{g BW}$) LPS.

Group III: (1 $\mu\text{g}/100\text{g BW}$)

Group IV: (10 $\mu\text{g}/100\text{g BW}$) LPS.

Group V: (100 $\mu\text{g}/100\text{g BW}$) LPS.

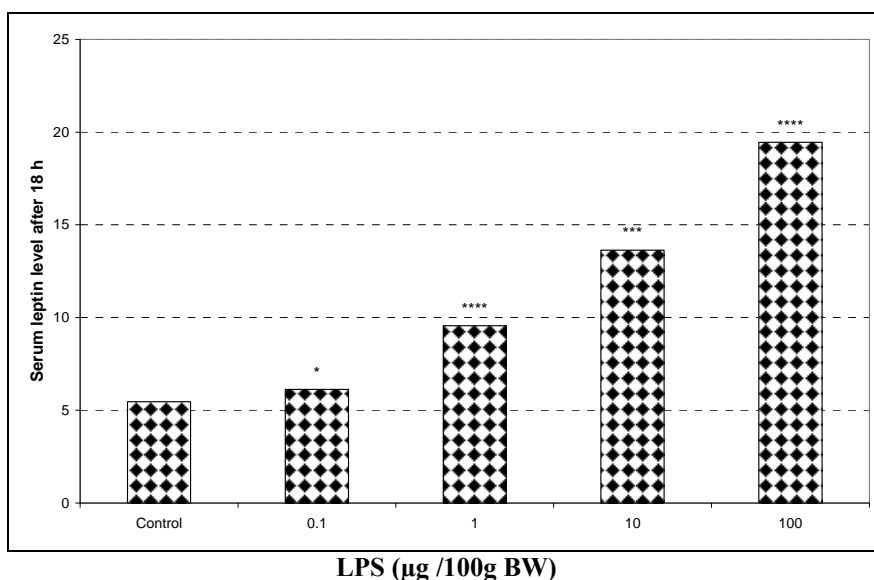


Fig.(3): Serum leptin levels (ng/ml) 18 h after LPS injection ($\mu\text{g} / 100\text{g BW}$) in control and LPS injected groups.

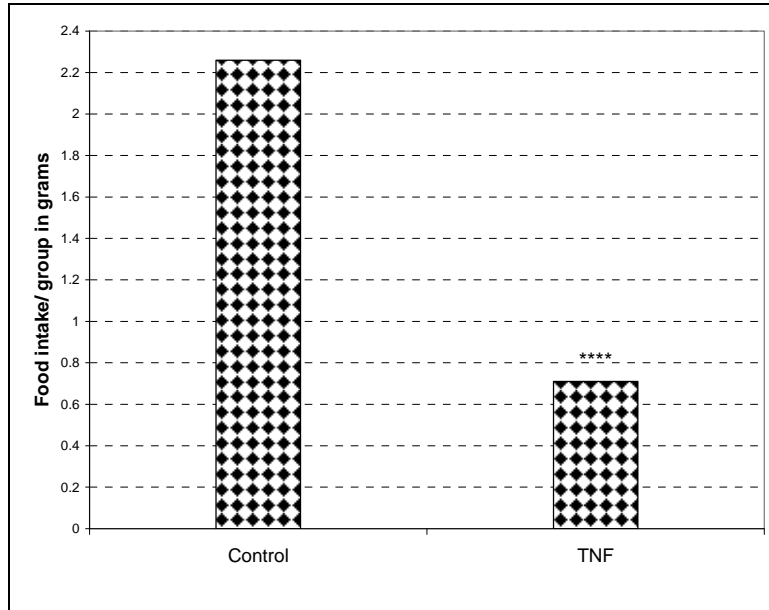


Fig.(4): Effect of $TNF-\infty$ on total food intake (in grams) by group VI, 18 h after injection in comparison with control group.

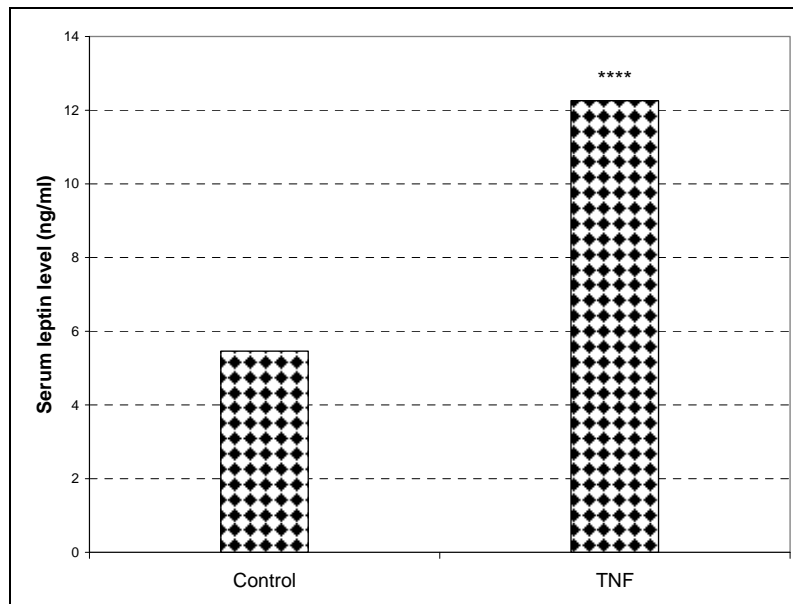


Fig.(5): Comparison of serum leptin level ($\mu\text{g/ml}$) in control group (I) and $TNF-\infty$ injected group (VI) 18 h after injection of $TNF-\infty$.

DISCUSSION

Leptin, a product of the anti-obesity gene is considered a major player in the regulation of body fat and has a lipo-atrophic effect^(21,22). Recent studies had showed increased leptin production during infection and inflammation providing a good evidence for the involvement of leptin in immunity and inflammation⁽²³⁾. Also, it has proved that leptin exerts a proliferative and anti-apoptotic activities in a variety of cell types⁽²⁴⁾.

Anorexia and weight loss are common manifestations of infections. Despite the presence of multiple metabolic changes during infection which may be attributed to be the cause of weight loss during infection, recent evidence indicates that decreased caloric intake due to anorexia plays a major role in infection-induced weight loss. For example, in AIDS, a state characterized by increased resting energy expenditure and disturbances in lipid metabolism, weight loss only occurs in the presence of decreased caloric intake⁽²⁵⁾.

Both gram negative and gram positive bacterial infections, are accompanied with severe anorexia. The actual reasons and mechanisms for that anorexia are still unknown. In experimental animals, bacterial infection can be induced by exposure to the bacterial cell wall product lipopolysaccharide (LPS) which causes a severe anorexia and weight loss few hours after exposure⁽²⁶⁾. In parallel, LPS leads to a rapid increase of serum leptin levels compared to fasted control animals⁽¹⁹⁾.

In the present study, a single intra-peritoneal (i.p.) injection of different doses of LPS to each mouse, (0.1µg, 1µg, 10µg and 100µg/100g. body weight), induced a significant ($p < 0.001$), dose dependent decrease in food intake and body weight 18 hour after injection (except for group II which showed a non significant decrease), as compared with control group. Mice injected with large doses of LPS (10 and 100µg/100g BW) showed marked and progressive decrease in food intake and weight loss during the whole period of the experiment (90 hour). These results were in agreement with those of Grunfed et al⁽¹⁹⁾, Langhans et al.⁽²⁷⁾, Basa et al.⁽²⁸⁾ Hick et al⁽²⁹⁾, Leininger et al.⁽³⁰⁾ and others who reported a significant decrease in food intake and body weight few hours after endotoxin administration to experimental animals. Also, Lugarini et al.⁽³¹⁾ revealed that high doses of LPS (500µ/kg or 1.0mg/kg) caused an initial (day 1) inhibition of feeding in rats. Also, the recovery of normal food intake was delayed after the highest dose (1.0 mg/kg) than that of the lowest dose.

Langhans,⁽³²⁾ reported that microbial products such as LPS trigger the acute-phase response (APR) and cause anorexia through induction of several pro-inflammatory cytokines which can act on some brain receptors to activate sickness-type behaviors including sleep, fever, general malaise and anorexia. TNF- α , IL-1, IL-2, interferon-gamma (IFN- γ) and ciliary neurotrophic factor (CNTF) are considered as most important cytokines that have been implicated in LPS anorexia⁽³³⁾.

TNF- α is the first cytokine that was held responsible for causing anorexia⁽³⁴⁾. As leptin production from adipose tissue is regulated by TNF- α and to a lesser extent by other cytokines, therefore, increased TNF- α during infection stimulates leptin secretion from adipose tissues. Increased serum levels of leptin hormone after infection lead to decreased food intake suggesting that leptin may contribute to the anorexia of infection⁽³⁾.

Although short-term anorexia may be beneficial for the host, long-term decrease in nutrient intake as in chronic infection decreases host defense, delayed recovery and is ultimately deleterious⁽³⁵⁾. Murray and Murray⁽³⁶⁾ reported a decrease in survival time and an increase in mortality after force-feeding in experimentally infected laboratory animals which reflects the short-term beneficial effect of anorexia during infection. As recorded by Klein et al⁽³⁷⁾, sick infected individuals should not be forced to eat unless in poor conditions as the anorexia associated with their infection is initially beneficial for them.

The suppression of hunger during infection in animals eliminates the need to find food, thus saving energy and reducing the heat loss from the body. In addition, anorexia reduces the availability of food-derived micronutrients such as iron and zinc that are essential for the growth of pathogenic microorganisms. Also, anorexia leads to premature death of infected cells. That conclusion was based on experimental animals data⁽³⁸⁾.

Güler et al.⁽³⁾ reported that apoptosis or cell suicide, is becoming recognized as a useful defense against intracellular pathogens, and nutrient restriction promotes apoptosis. It is of critical importance for infected cells to kill themselves rapidly before they can promote the spread of pathogens to other cells.

As LPS administration induces severe decrease in food intake and body weight in parallel to a rapid increase of serum leptin levels compared to fasted control animals, many investigators revealed that leptin may be a possible mediator of anorexia during infections^(19,32,37). In the present study, intra-peritoneal injection of different doses of LPS “0.1, 1, 10 and 100 $\mu\text{g}/100\text{g BW}$ ” leads to a significant increase in serum leptin levels 18h after injection in a dose related manner. These results were in agreement with the results of previous studies. Zhang et al.⁽³⁹⁾ reported that endotoxin administration increased serum leptin levels and leptin has been shown to act in an endocrine fashion to regulate food intake and body weight. Moshayed et al.⁽²⁾ recorded significant increase in serum leptin and ob gene mRNA levels in mice with bacterial peritonitis, peaking at 24h and declining to base-line thereafter, food intake was reduced similarly over the first 72h in these infected mice.

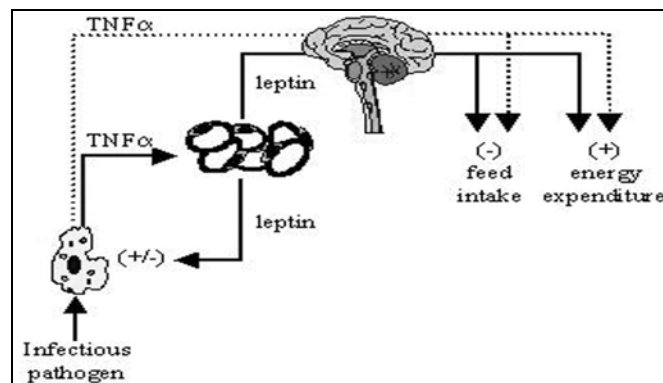
Guyon et al.⁽²⁶⁾ reported that LPS is known to increase leptin synthesis and secretion at the periphery. Then, leptin is transported into the brain where it decreases appetite and increases body temperature. Leptin is able to modulate in a coordinated manner the activity of neurons of the

hypothalamus expressing leptin receptors, stimulating neurons of the arcuate nucleus producing anorexigenic peptides such as pro-opiomelanocortin (POMC) neurons and inhibiting neurons producing orexigenic peptides such as neuropeptide Y (NPY) neurons. Also, the weight reducing effect of leptin can be due to increased catecholamines release in response to increased serum leptin⁽⁴⁰⁾. Catecholamines could act on adipocytes to cause lipolysis. Leptin also, causes fat cell apoptosis⁽⁴¹⁾.

Neutralization of circulating leptin with leptin antiserum has recently been shown to block the feeding inhibitory effect of LPS, suggesting that leptin is a circulating mediator of LPS anorexia⁽⁴²⁾.

Role of leptin in regulating food intake during infection is much less clear. Sarraf⁽⁴⁾ and Grunfeld⁽¹⁹⁾ suggested that during acute inflammation, pro-inflammatory cytokines such as TNF- α and IL-1 may induce anorexia and weight loss via induction of leptin. During infection, there is marked increase in cytokines secreted by leukocytes as

IL-1, IL-6 and TNF- α .⁽⁴³⁾ These cytokines are secreted during immune challenge, and many studies have shown that injection of TNF- α or IL-1 reduced food intake even in absence of any infection^(19,37). Moreover, these cytokines and their receptors are present in the brain, and inhibiting their secretion or blocking their receptors in the brain has been shown to block anorexia induced by inflammatory stimuli or infection. These results suggested that anorexia of infection is mediated by direct central action of cytokines in the brain. Also, during infection, cytokines can act peripherally on adipose tissues by direct stimulation of adipocytes to increase leptin production which is another way by which immune system may transmit a message to the brain to decrease food intake during infection **(Fig. 6)**. So, cytokines allow the immune system to “speak” to other physiological systems. Also, such interplay between immune cells and fat cells is considered to be an example of how different physiological systems are cooperated specially during illness⁽⁴⁴⁾



(Fig. 6)⁽⁴⁴⁾

In the present study, the effect of TNF- α injection on leptin secretion without LPS infection was studied. 18 h after i.p. injection of group VI by TNF- α in a dose of 17 μ g/100g BW, the treated group animals showed a significant reduction in food intake and a significant increase in serum leptin levels when compared to the control group ($p < 0.001$). These results were in agreement with those of Grunfeld et al.⁽¹⁹⁾ Sarraf et al.⁽⁴⁾ and Finck et al.⁽⁴⁵⁾ who demonstrated that injection of TNF- α induced a reduction in food intake and stimulates leptin synthesis from adipocytes and increases leptin level in serum. Also, previous studies have shown that the anorectic effects of LPS were partly mediated via cytokines such as TNF- α ⁽⁴⁶⁾.

Grunfeld et al.⁽¹⁹⁾ reported that the host response to infection is primarily mediated by cytokines of the immune system, in particular TNF- α and IL-1. They confirmed in a study on hamster that TNF- α induced anorexia, increased leptin mRNA levels in adipose tissue and leptin protein in circulation. Also, from their studies they suggested that leptin induced by LPS and TNF- α directly contributes to the anorexia seen during infection. Moshayed et al.⁽²⁾ confirmed that there is a transient increase in the plasma leptin response to bacterial infection, peaking at 24h and declining to baseline thereafter and that inhibition of TNF- α activity attenuates that response.

Conversely, other studies reported that leptin can in fact up-regulate LPS-induced TNF- α production, as leptin can regulate the pro-inflammatory responses and

stimulate proliferation and activation of human circulating monocytes after infection^(47,48,49). Although cytokines have been shown to acutely increase leptin levels after LPS infection in humans^(50,51), LPS does not appear to modulate circulating leptin levels after inhibition of TNF- α by anti-inflammatory agents⁽⁵²⁾.

TNF- α is a pro-inflammatory cytokine produced mainly by mononuclear phagocytic cell of the immune system. It was discovered in two different areas of biology in the mid-1980. It was first isolated as a factor that caused tumor necrosis in experimentally infected animal^(53,54,55). Other studies identified the ability of that factor to reduce body weight⁽⁵⁶⁾. More recently TNF- α is considered the first cytokine to increase leptin production from the adipose tissue. LPS injection leads to anorexia and increase serum leptin. Also, TNF- α and other pro-inflammatory cytokines are increased after LPS. To determine whether the induction of TNF- α by LPS is involved in the increased plasma leptin levels after challenge with LPS, Finck et al.⁽⁴⁵⁾ studied the effects of LPS and TNF- α on leptin secretion in both LPS-sensitive mice (OuJ mice) and in LPS insensitive (HeJ) mice. After LPS injection OuJ mice secreted cytokines and thus responded normally to LPS injection, whereas HeJ mice are not sensitive to LPS and do not secrete cytokines after LPS injection. By measuring serum TNF- α and leptin levels after LPS injection in both groups, they recorded increased serum TNF- α and leptin in sensitive OuJ mice. However, in LPS insensitive mice, LPS induced neither TNF α nor leptin, suggesting a

possible relationship between the induction of TNF- α and leptin. Moreover, *in vitro* studies with primary adipocytes cultured from OuJ and HeJ mice showed that TNF- α but not LPS, increased supernatant leptin levels in both mice groups. These results clearly indicate that TNF- α can induce leptin secretion by direct interaction with adipocytes⁽⁴⁵⁾.

Several studies have reported a high positive correlation between TNF- α and leptin in plasma. For instance, mice that lack TNF- α are obese and have lower basal plasma leptin levels⁽⁴⁴⁾. Collectively, these data suggest that leptin may interact with TNF- α to mediate LPS-induced anorexia.

It has been proved that inhibition of TNF- α activity significantly reduced peak plasma leptin levels 18-24 h after LPS injection in mice. Also, Porter et al,⁽⁵⁷⁾ reported that inhibition of TNF- α production by pentoxifylline, for example, eliminated LPS induced anorexia. Several other studies indicated that anti-inflammatory agents inhibit leptin induction by TNF- α ^(58,59,60,61). Finck et al.⁽⁴⁵⁾ demonstrated that culture with TNF- α inhibitors as actinomycin-D blocked the induction of leptin following TNF- α . Explants were also cultured in the presence of the anti-inflammatory as prostaglandin J₂ (PGJ₂) and then exposed to TNF- α . That compound completely abolished TNF- α induced increases in leptin production. Also, mice pretreated with PGJ and then given TNF- α , PGJ treatment markedly blunted the TNF- α induced increase in leptin. Collectively, these data indicate that

TNF- α acutely activates leptin expression and that anti-inflammatory agents can block TNF- α induced hyperleptinemia.

Recent studies indicate that leptin activates the immune system. Also, they suggest that leptin has anti-inflammatory properties as well⁽⁴⁴⁾. Because leptin shares significant sequence and structural homology with interleukin-6 (IL-6) family of cytokines⁽³⁹⁾ and their receptors⁽⁴⁹⁾, it is now generally accepted to be both an endocrine hormone and a member of the class 1 cytokine group^(62,63)

Immunologic properties of leptin have been revealed by many investigators. Gainsford et al,⁽⁶⁴⁾ found that addition of leptin to culture medium enhanced cytokine production and phagocytosis of *Leishmania* parasites by peritoneal macrophages. This was again demonstrated in another studies^(47,65) that leptin increased phagocytic activity of macrophages and potentiated TNF- α , IL-6 and IL-12 secretion in response to LPS *in vitro*. Leptin might also, significantly influence acquired immunity because it was shown to stimulate memory T-Cell maturation.⁽⁴⁴⁾

The mechanisms of the anorexia during infection are not yet fully understood, but are presumably based on interactions between various cytokines and other humoral mediators of the effects of microbial products.⁽³⁾ LPS infections increase cytokines production, which can affect food intake by several pathways as reported by Guyon et al, 2008.⁽²⁶⁾ Cytokines can act peripherally on adipose tissues producing hormones such as leptin which influence the

activity of the brain for food intake by many ways as mentioned before. Also, cytokines can directly enter the brain as the permeability of the blood brain barrier (BBB) is increased during LPS infection, then, they act locally on different neurons concerning with food intake.⁽⁶⁶⁾

Basa et al.⁽²⁸⁾ revealed that LPS injections to rats decreased ghrelin hormone levels in fasted rats compared to control levels. Ghrelin is a peptide hormone produced by the stomach with a potent orexigenic effect. IL-1, TNF- α and prostaglandins are considered as the most important cytokines by which LPS suppresses plasma ghrelin.⁽⁶⁷⁾

Another mechanism by which infection induces anorexia is through MCH neurons (melanin-concentrating hormone). As reported by Sergeev et al.⁽⁶⁸⁾ cytokines induction following LPS injection in mice, lead to a decrease in expression of MCH mRNAs. The decrease in MCH release from MCH neurons of the lateral hypothalamus could lead to anorexia. Therefore, infection can lead to variation in the concentration of various hormones and cytokines in the blood which can cross the BBB and act directly on the brain to induce anorexia during infection.

Langhans,⁽³²⁾ reviewed that LPS or TNF- α treatment increased serotonergic projection from midbrain raph area and the hindbrain to the hypothalamus. Also, it has been found that serotonin (5-HT) cells accumulating in these area possess PG EP₃ receptors and are activated by PG E₂. Also, administration of TNF- α or IL-1 increased serotonergic activity in dorsal raph nucleus⁽⁶⁹⁾. It is well

known that serotonin is a potent inhibitor of eating mainly through the 5-HT_{2c} receptors in the hypothalamus. Recent evidence indicates that serotonin modulates the release of endogenous agonists and antagonists of brain melanocortin receptors (MC_n-R), which are crucial for the central control of energy balance⁽⁷⁰⁾. But although several findings implicate the 5-HT neurons in median raph nucleus and 5-HT_{2c} receptors in the hypothalamus in LPS anorexia, it is not yet clear whether that mechanism plays a necessary role in anorexia during infection⁽³²⁾.

Another possible mechanism for anorexia during infection is through orexin-A protein. Recent findings⁽⁷¹⁾ suggest that peripheral LPS decreases the numbers of lateral hypothalamic area neurons expressing orexin-A protein in mice. As orexin-A has a potent orexigenic effect and arousal effect⁽⁷²⁾, the inhibitory affect of LPS on orexin-A protein-expressing neurons might, also, be involved in LPS-induced anorexia and decreased activity in infected animals. Collectively, increased understanding of the cooperation between immune activation mediators (cytokines) and hormonal changes during infections could lead to further studies of the host's immune and metabolic response during infection^(23,73).

In conclusion:

The present study showed that both of LPS and TNF- α can induce anorexia and increased leptin level. The decrease in food intake is inversely proportional to the increase in serum leptin. These data suggest a role for leptin in anorexia during LPS infection. So, the present study is

strongly advance the hypothesis that cytokine induction of leptin during infection plays a significant role in occurrence of anorexia. The study, also, explained some possible mechanisms for anorexia during infection through the cooperation between immune activation mediators (cytokines) and some hormonal changes which can induce different host's immune and metabolic response during infection.

Given the prominent role that anorexia plays in inflammatory diseases such as chronic infection, collagen disease, cancer and wasting syndrome, it is possible that development of leptin antagonists may play a useful role in decreasing anorexia and wasting of chronic infections such as AIDS.

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دور كل من هرمون الليبتين ومعامل تنخر الورم - ألفا في آليات فقدان الشهية أثناء العدوى بالسموم الداخلية البكتيرية في الفئران

خلفية البحث : فقدان الشهية وفقدان الوزن هي السمة المميزة للعدوى. الآليات الفعلية التي تسبب بها العدوى فقدان الشهية لا تزال غير معروفة. ولقد اثبتت العديد من وسطاء المناعة ماقبل الالتهاب (proinflammatory cytokines) - و أبرزها عامل تنخر الورم- ألفا (TNF- α) - المعروفة بقدرتها علي بدء وتعديل استجابة العائل للعدوى، قدرتها علي التسبب في فقدان الشهية وفقدان الوزن في الحيوانات السليمة. هرمون الليبتين هو هرمون بروتيني تنتجه الأنسجة الدهنية يعتبر عاملا مضاد للتخمة حيث انه يخفض من كمية الاستهلاك الغذائي، ويزيد من استهلاك الطاقة. ولقد اظهر تعاطي كل من عامل تنخر الورم - ألفا والسموم الداخلية للبكتيريا زيادة هرمون الليبتين في مصل الدم ولقد ثبت ان الليبتين يعمل بطريقة هرمونية لخفض الاستهلاك الغذائي و وزن الجسم. وهذا يشير إلى أنه ربما يكون الليبتين واحدا من الآليات المسببة لفقدان الشهية أثناء العدوى.

تهدف هذه الدراسة إلي بحث تأثير السموم الداخلية (LPS) و عامل تنخر الورم-ألفا (TNF- α) علي معدل الاستهلاك الغذائي، ووزن الجسم ومستويات مصل الليبتين في الفئران. أيضا تحاول الدراسة شرح لمختلف الآليات الممكنة التي يمكن أن تسبب فقدان الشهية ، وإذا كان الليبتين له دور في هذه الآليات.

الطريقة : أجريت الدراسة على ٣٦ من إناث الفئران البالغة التي قسمت إلى ٦ مجموعات ، كل مجموعة ستة فئران، تم الحقن بريوتونيا لكل المجموعات. المجموعة الأولى : "مجموعة ضابطة" التي تم حقنها بمحلول ملحي عادي. المجموعة الثانية ، والثالثة والرابعة والخامسة. تم حقنها بجرعات مختلفة من ال (LPS) كالآتي:

١، ٠، ١، ١٠ و ١٠٠ / ميكروجرام / ١٠٠ جرام من وزن الجسم علي التوالي . المجموعة السادسة تم حقنها ب (TNF- α) بجرعة ١٧ ميكروجرام / ١٠٠ جرام من وزن الجسم. تم قياس الاستهلاك الغذائي ووزن الجسم بعد ١٨ ، ٤٢ ، ٦٦ و ٩٠ ساعة من الحقن للمجموعة الضابطة ومجموعة ال LPS . اما بالنسبة لمجموعة ال (TNF- α) فقد تم حساب كمية الاستهلاك الغذائي بعد ١٨ ساعة من الحقن فقط. أيضا، تم سحب عينة دم من فئران كل المجموعات بعد ١٨ ساعة من الحقن وتم فصل المصل ثم قياس نسبة هرمون الليبتين في الدم باستخدام اختبار الأليزا.

النتائج : أظهرت الدراسة انخفاضا (متزايدا مع زيادة جرعة الحقن) في الاستهلاك الغذائي ووزن الجسم في كل المجموعات المحقونة ب ال (LPS) و ذلك بالمقارنة بالمجموعة الضابطة. كما لوحظ أيضا أن الانتعاش التدريجي في الاستهلاك الغذائي والزيادة في وزن الجسم على مدى الثلاثة أيام التالية للحقن قد حدث في المجموعات المعاملة بجرعات منخفضة (المجموعتين الثانية والثالثة) ، أما الفئران المعاملة بجرعات كبيرة ، (المجموعتين الخامسة والسادسة) ، لم تظهر أي تحسن في معدل الاستهلاك الغذائي أو زيادة الوزن خلال ال ٤٢ ساعة التالية للحقن. بعد ذلك ، بدأت المجموعة الرابعة في زيادة الاستهلاك الغذائي ووزن الجسم حتى نهاية الدراسة ، بينما ظلت المجموعة الخامسة في حالة فقدان تام للشهية ونقص متزايد في وزن الجسم حتى نهاية الدراسة. المجموعة التي تم حقنها ب (TNF- α) أظهرت انخفاضا كبيرا في الاستهلاك الغذائي بعد مرور ١٨ ساعة علي الحقن و ذلك بالمقارنة مع المجموعة الضابطة ، وهذا الانخفاض في الاستهلاك الغذائي بعد حقن (TNF- α) كان تقريبا مشابها لذلك الذي أحدثه حقن ال (LPS) . كما أظهرت مستويات الليبتين بالمصل زيادة كبيرة بعد ١٨ ساعة من حقن كل من (LPS) و (TNF- α) في جميع المجموعات و ذلك بالمقارنة بالمجموعة الضابطة ، هذه الزيادة أظهرت علاقة ارتباط إيجابية مع جرعة الحقن في مجموعات ال (LPS) ، ولقد أظهرت المجموعة الخامسة التي تم حقنها بأعلى جرعة لل (LPS) زيادة بحوالي أربعة أضعاف نسبة الليبتين في المجموعة الضابطة. اما الزيادة في مستويات الليبتين بالمصل بعد حقن (TNF- α) فهي لم تصل الي نفس مستويات الزيادة بعد حقن (LPS) خاصة للجرعات العالية من ال (LPS) كما في المجموعة الرابعة والخامسة.

وختاما : هذه الدراسة تبين أن كلا من السموم الداخلية (LPS) و (TNF- α) يؤدي إلى فقدان الشهية وزيادة مستوى الليبتين في مصل الدم، كما أن النقص في الوجبات الغذائية يتناسب تناسباً عكسياً مع زيادة في نسبة الليبتين في السيروم. و بذلك قد تشير هذه البيانات إلى وجود دور لليبتين في فقدان الشهية من خلال العدوى بال (LPS). ولقد كشفت هذه الدراسة عن بعض الآليات المسببة لحدوث فقدان الشهية ونقص الوزن أثناء العدوى وذلك من خلال تفعيل التعاون بين وسطاء المناعة (cytokines) وبعض التغيرات الهرمونية التي تؤدي إلى تغيرات مختلفة في مناعة العائل و رد الفعل الأيضي أثناء العدوى. و نظرا للدور الحيوي الذي يقوم به فقدان الشهية أثناء أمراض الالتهابات المزمنة، فإنه من الممكن تطوير مضادات لهرمون الليبتين والتي قد تلعب دورا مفيدا في الحد من فقدان الشهية والهزال المزمن المصاحب للعدوى المزمنة مثل حالات الإصابة بمرض الإيدز.