

## **EFFECT OF CHRONIC LEPTIN TREATMENT AND EXERCISE ON BODY WEIGHT, SERUM GLUCOSE AND INSULIN LEVELS OF SPRAGUE-DAWLEY RAT**

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### **Abstract**

Leptin is known to increase glucose metabolism and energy expenditure while insulin increases glucose uptake into peripheral tissue. However, in obese and type 2 diabetes mellitus (T2DM) patients, high levels of both leptin and insulin are observed, indicating resistance to these two hormones in these individuals. It is unclear if elevated leptin levels are the cause of insulin resistance in these individuals. Physical exercise is known to be useful in the management of obesity and T2DM. It has been found that exercise can decrease bodyweight and increase glucose uptake in these individuals following exercise. This study aims to determine the effect of chronic leptin treatment and exercise on body weight, serum glucose and insulin levels of Sprague-Dawley rat. Eight-week old rats were treated with either intraperitoneal injection of normal saline (Control; n=8), or leptin (60 µg/kg body weight/day; Leptin; n=8), or leptin and exercise (60 µg/kg body weight/day plus running on a treadmill every other day for 30 minutes at a speed of 30 m/min with 10° inclinations; Leptin-exercise; n=8) or exercise only (running every other day for 30 minutes at a speed of 30 m/min with 10° inclinations on a treadmill; Exercise; n=8) for six weeks. Following six weeks of treatment, glucose challenge was performed by intravenous infusion of 100 mg/ml of glucose for 5 minutes. During the protocol, blood was drawn at 0, 5, 10, 15, 20, 25 and 30-min for estimation of serum glucose, and serum insulin levels. Data were analyzed using one-way ANOVA with post-hoc analysis and expressed as mean ± standard error of mean (SEM). Despite not statistically different in body weight between the groups, leptin group shows higher trend of mean body weight indicating treatment with leptin might induce leptin resistance in this group. Moreover, glucose clearance was also delayed in this group showing decreased insulin action associated with lower insulin level in leptin group. More importantly, exercise reversed the leptin effects by promoting glucose clearance. In conclusion, six weeks of daily leptin administration resulted in delayed glucose clearance, however, concurrent exercise prevented these effects of leptin by promoting glucose clearance signifying increase in insulin action.

**Keywords:** leptin, glucose challenge, insulin

### **INTRODUCTION**

Glucose concentration in a healthy individual range between 5 and 6 mmol/l (David & John, 2014). It is crucial to maintain glucose homeostasis since it is primarily ensuring an adequate energy supply to organs that rely on glucose as their energy source such as brain, and for protecting the blood vessels from damage due to dangerously high level of glucose (Röder, Wu, Liu & Han, 2016). Glucose homeostasis is usually achieved by stimulating glucose disposal into muscle and adipose tissue and

inhibiting hepatic glucose production after glucose and other fuels enter the body following a meal. This is achieved through the interplay of numerous glucose-regulating hormones. The primary hormone in charge of glucose homeostasis is insulin. Insulin is necessary for the uptake of glucose by various tissues in the body, including skeletal muscle and adipose tissue. Insulin mediates its biological effects through its interaction with the cell surface insulin receptor (IR). The binding of insulin to insulin receptor results in activation of intracellular signaling pathways that can lead to glucose uptake and/or hepatic glucose production through gluconeogenesis (Leto & Saltiel, 2012; De Meyts, 2016). Development of insulin resistance and altered glucose homeostasis can be observed by tissue-specific IR knockout in mice and similar impairments have been often seen in diabetic patients (Brüning et al., 1998; Blüher et al., 2002; Cignarelli et al., 2019).

Adipose tissue secretes numerous adipokines particularly leptin and it is directly related to the amount of adipose tissue (Blüher & Mantzoros, 2015). Leptin is a 16-kDa obese (*ob*) gene product that acts centrally via a negative feedback signal on the hypothalamus, which in turn attempts to prevent excessive weight gain by decreasing appetite, reducing food intake and increasing energy expenditure (Katherine, Alexandra & Jessica, 2019). However, high leptin levels have been reported in most overweight and obese individuals, indicating central resistance to endogenous leptin in human obesity (Sari, Balci, & Apaydin, 2010; Ekmen, Helvacı, Gunaldi, Sasani & Yildirmak, 2016). Moreover, evidence shows that leptin may be independently involved in the regulation of insulin secretion and action (Cantley, 2014). Reports also show that prevalence of insulin resistance is high in obese individual with high leptin levels (Sáinz, Barrenetxe, Moreno-Aliaga, & Martínez, 2015). Thus, leptin resistance or hyperleptinemia could be a 'key link' between obesity and insulin resistance in T2DM.

Physical exercise is known to be useful in the management of diabetes mellitus (Melmer, Kempf & Laimer, 2018). Several studies have demonstrated that exercise improves insulin signaling in the muscle (Sjøberg et al., 2017). However, the effects of exercise on leptin levels are conflicting. Investigators had reported that leptin levels fall depending on the duration, intensity and caloric cost of exercise (Bouassida et al., 2010). Moreover, T2DM individuals also show reduction in leptin levels following exercise independent of change in body fat mass (Ishii et al., 2001). In contrast, others had reported that there is no change in leptin levels after exercise (Bouassida et al., 2004; Nuri, Moghaddasi, Darvishi & Izadpanah, 2016). Furthermore, insulin sensitivity increases in skeletal muscle tissues following exercise, indicating a possible mechanism for the effects of exercise on glucose metabolism (Sjøberg et al., 2017; Bird & Hawley, 2017). Additionally, acute exercise also improves insulin sensitivity in hypothalamus and restores food intake control in obesity (de Almeida et al., 2014). Studies also show that exercise increases glucose utilization and uptake by peripheral tissues (Bradley et al., 2014). T2DM patients also show better glucose tolerance and insulin action following exercise training (Way, Hackett, Baker, & Johnson., 2016).

Although it appears that high leptin levels might contribute to the development of insulin resistance and T2DM in obese subjects, the association between leptin and insulin resistance is however not fully understood. Whether the impact of physical exercise on glucose utilization in hyperleptinaemia of obesity is associated with alterations in the leptin and insulin interaction is also unknown. Therefore, this study investigates the effect of chronic leptin treatment and exercise on body weight, glucose and insulin levels following glucose loading in rat. The findings will highlight the interaction of leptin and exercise on body weight, glucose homeostasis and insulin action.

## **METHODOLOGY**

### **Animals and experimental procedures**

Male Sprague-Dawley rats (250-300 grams), aged 8-10 weeks were divided into four groups; control (n=8), leptin-treated (leptin; n=8), leptin-treated exercise (leptin-exercise; n=8), and exercise only (exercise; n=8). They were housed in a room maintained on 12:12 hours light cycle at room temperature and allowed free access to water and rat chow. Leptin (BioVision, USA) was injected intraperitoneally (i.p.) at 60 µg/kg body weight in 0.1 ml normal saline daily to leptin-treated rats.

Exercise was done in every other day for 30 minutes at a speed of 30m/min with 10° inclinations on a treadmill (Columbus, USA). Control group was given 0.1 ml of normal saline daily. All treatment went on for 6 weeks. Body weights were measured once a week. The Institute's Animal Care and Users Committee, Faculty of Medicine, Universiti Teknologi MARA approved all procedures used in the experiment.

### **Exercise procedure**

The training session were performed during the light cycle and consisted of thirty minutes running sessions, every other day for six weeks on a three-track treadmill especially designed for small animals like mice and rats. Three animals were exercised each time and all exercise was performed at the same time of the day. Volume and intensity of training and electric stimulus (repetition rate and intensity of the electric shock) were gradually increased until the rats ran for thirty minutes at 30 m/min with 10° inclinations on the treadmill. Thereafter, volume and intensity were constant. The exercise was always observed during the procedure to ensure the safety of the exercised animals.

### **Glucose tolerance test procedure**

Twenty-four hours after the last session of training, as indicated in the time course experiments, the exercised rats and the other groups were submitted to a glucose tolerance test (100 mg/ml of glucose), after 16 hours fasting with free access to water. Briefly, the rats were anaesthetized with intraperitoneal (i.p.) injection of thiopental (Sandoz, Austria) (70 mg/kg body weight). The anaesthetized rats were then placed on a heating pad (Harvard apparatus, UK) that was maintained at 37°C. As soon as anesthesia was assured by the loss of pedal and corneal reflexes, the ventral cavity was opened, right jugular vein and left carotid artery were exposed and cannulated for glucose infusion and blood sampling respectively. A baseline blood sample was collected from artery into a 1.5 ml tube for both glucose and insulin estimations. Then 100 mg/ml glucose was immediately infused intravenously (i.v.) into the vein at a rate of 100 µl/min for five minutes (Braintree Scientific, USA). Blood samples were collected at 0 (baseline), 5 (glucose load), 10, 15, 20, 25 and 30 minutes of post glucose administration for serum glucose and insulin determination.

### **Serum glucose and insulin quantification**

Approximately 1 ml of blood was withdrawn in a dry 1.5 ml tube without anticoagulant during the glucose tolerance test procedure. Blood samples were allowed to clot in ice and then serum was separated by centrifugation at 3200 rpm for 6 minutes to collect serum aliquots that were stored at -80°C until assay. Commercially available quantitative sandwich enzyme linked immunosorbent assay (ELISA) kits (Mercodia, Sweden) was employed to measure serum insulin levels. Each sample was assayed in duplicate along with standards and quality control sera with every assay run. The sensitivity of the assay is  $\leq 0.15$  µg/L. The data was extrapolated using a standard curve graph.

### **Statistical analyses**

All the parameter measured was expressed as mean  $\pm$  standard error of mean (SEM). Statistically significant differences between the control and test groups for the study were done using one-way analysis of variance (ANOVA) and post-hoc analysis was done to compare individual groups. The differences were considered significant at  $p \leq 0.05$ .

## RESULTS

### Body weight

A one-way between-groups analysis of variance was conducted to explore the impact of 6-week of leptin treatment and exercise on body weight. There was no statistically significant different found. The means and standard error of mean are presented in Table 1. Nevertheless, leptin group has slightly higher mean body weight starting from week 3 when compared to the other groups.

**Table 1.** Mean Body Weight (in grams) of Control and Leptin Treated Rats

Group	Body Weight (g)						
	0	1	2	3	4	5	6
Control	267 ± 4.2	294 ± 3.3	320 ± 4.6	341 ± 5.6	356 ± 7.9	369 ± 7.2	368 ± 8.7
Leptin	267 ± 3.5	297 ± 3.9	327 ± 3.1	351 ± 4.0	376 ± 4.8	389 ± 10.0	375 ± 13.6
Leptin-exercise	270 ± 4.2	289 ± 4.7	316 ± 5.5	337 ± 7.3	353 ± 7.9	365 ± 7.2	358 ± 7.2
Exercise	270 ± 4.9	294 ± 6.7	318 ± 8.7	339 ± 7.7	353 ± 8.6	366 ± 8.1	359 ± 7.4

### Serum glucose levels

There was a statistically significant difference at the  $p < 0.05$  level at time 30 minutes:  $F(3, 20) = 3.4$ ,  $p = 0.03$ . The actual difference in mean scores between the groups was quite large. The effect size, calculated using eta squared, was 0.33. Post-hoc comparisons using the Tukey HSD test indicated that the mean score for leptin group ( $M = 9.18$ ,  $SEM = 0.65$ ) was significantly different from leptin-exercise group ( $M = 6.62$ ,  $SEM = 0.19$ ). Control group ( $M = 6.90$ ,  $SEM = 0.55$ ) and exercise group ( $M = 7.03$ ,  $SEM = 0.94$ ) did not differ significantly from either leptin-treated groups at time 30 minutes.

**Table 2.** Serum Glucose Levels (in mmol/l) of Control and Leptin and Exercise Treated Rats during Glucose Challenge

Group	Serum Glucose level (mmol/l)						
	0	5	10	15	20	25	30
Control	7.53 ± 0.07	14.23 ± 0.38	10.02 ± 0.17	9.22 ± 0.51	8.77 ± 0.41	8.10 ± 0.42	6.90 ± 0.55
Leptin	7.35 ± 0.33	13.57 ± 0.41	9.92 ± 0.39	9.13 ± 0.44	9.52 ± 0.48	9.43 ± 0.53	9.18* ± 0.65
Leptin-exercise	6.95 ± 0.40	13.47 ± 0.40	9.70 ± 0.28	8.80 ± 0.40	8.83 ± 0.27	8.63 ± 0.11	6.62* ± 0.19
Exercise	6.97 ± 0.28	12.97 ± 0.44	9.63 ± 0.27	9.02 ± 0.28	8.85 ± 0.25	7.92 ± 0.65	7.03 ± 0.94

\*:  $P < 0.05$  when compare leptin group with leptin-exercise group at time 30-min

### Serum insulin levels

There was a statistical significant difference at the  $p < 0.05$  level at time 5 minutes:  $F(3, 26) = 4.76$ ,  $p = 0.009$ . the actual difference in mean scores between the groups was quite large. The effect size, calculated using eta squared, was 0.35. Post-hoc comparisons using the Tukey HSD test indicated

that the mean score for leptin-exercise group ( $M = 2.19$ ,  $SEM = 0.37$ ) and exercise group ( $M = 2.61$ ,  $SEM = 0.49$ ) were significantly different from control ( $M = 4.77$ ,  $SEM = 0.67$ ). Leptin group ( $M = 2.94$ ,  $SEM = 0.58$ ) did not differ significantly from either groups at time 5 minutes.

**Table 3.** Serum Insulin Levels (in  $\mu\text{g/l}$ ) of Rats During Glucose Loading

Group	Serum Insulin Levels (in $\mu\text{g/l}$ )					
	0	5	10	15	20	30
Control	1.04 $\pm$ 0.13	4.77 $\pm$ 0.67	2.11 $\pm$ 0.63	2.52 $\pm$ 0.64	2.66 $\pm$ 0.61	3.46 $\pm$ 0.73
Leptin	0.92 $\pm$ 0.21	2.94 $\pm$ 0.58	1.33 $\pm$ 0.27	1.24 $\pm$ 0.29	2.20 $\pm$ 0.68	2.97 $\pm$ 0.78
Leptin-exercise	0.62 $\pm$ 0.11	2.19** $\pm$ 0.37	1.41 $\pm$ 0.44	1.68 $\pm$ 0.65	2.04 $\pm$ 0.60	2.37 $\pm$ 0.53
Exercise	1.03 $\pm$ 0.27	2.61* $\pm$ 0.49	1.29 $\pm$ 0.16	1.92 $\pm$ 0.45	2.34 $\pm$ 0.69	3.01 $\pm$ 0.31

\*:  $P < 0.05$ ; \*\*:  $P < 0.01$  when compared to control at time 5-min

## DISCUSSION

### Body weight

The results from the study showed there were no differences in the body weight between the groups. Despite the role of leptin in regulating energy homeostasis and metabolism, there are several other possible reasons that might explain the lack of leptin action on body weight in this study. Leptin has a short half-life of about 9 to 12 minutes in the rat circulation (Zeng et al., 1997) and 43 minutes in mouse (Burnett, Skowronski, Rausch, LeDuc & Leibel, 2017). Its effects on food intake might therefore only last for a short duration and this may not get reflected in a 24-hour energy balance. Furthermore, a rebound increase in food intake has been reported right after a short duration of suppression of food intake (fasting or reduced satiety) (Kumar, Shimokawa, Nagy, & Lane, 2002). This might counteract the effect of leptin on food reduction and explain the lack of any differences in body weight in controls and leptin treated groups in this study. The dose of leptin administered was 60  $\mu\text{g/kg}$  body weight daily via intraperitoneal route for six weeks. Other studies with doses of 5, 10, 30 and even 120  $\mu\text{g/kg}$  body weight daily of leptin administration have also been shown not to have an effect on body weight (Haron, D'Souza, Jaafar, Zakaria, & Singh, 2010; Ibrahim, Omar, Ruth, Froemming, & Singh, 2013). The dose was primarily chosen as to avoid significant differences in body weight from appearing, which could by itself contribute to changes in glucose metabolism and insulin sensitivity. Significant differences in body weight in leptin and non-leptin treated rats would unnecessarily add another variable. There was also no significant difference in body weight between the exercised and non-exercised rats (Lalanza et al., 2015). Kang et al. (2013) supported this finding by which no significant difference was found in body weight of obese rats that were exercised for 8-week on a treadmill for 40 minutes once a day for 7 days. Whilst exercise is known to increase energy expenditure and consequently reduce body weight, its effect on body weight might also be influenced by food intake. A concomitant increase in food intake with exercise is therefore unlikely to cause a decrease in body weight. It is possible that this might have been the case here too. Food intake was not measured in this study and it is not possible to say if the lack of difference in body weight between the groups was due to increased food intake in the exercised groups. Moreover, a combination of diet restriction and exercise timing (eating before exercise) could have caused less body fat weight gain and more skeletal muscle weight (Sasaki, Ohtsu, Ikeda, Tsubosaka, & Shibata, 2014). Nevertheless, of more importance in this study is the fact that any effect of leptin or exercise on glucose metabolism would therefore be a direct effect of either leptin or exercise and not an indirect effect through changes in body weight.

## **Serum glucose levels**

Glucose challenge was done to determine the effect of leptin treatment on glucose utilization in skeletal muscle of exercised and non-exercised rats. A mean fasting serum glucose concentration of 7 mmol/l recorded before the glucose challenge is similar to values observed in lean mice treated with 10 µg/day leptin infusion for 7 days (Harris, 1998) and within the normal range of 5 to 7 mmol/l in the same species (Chen, Chen, Qian, Jiang, & Chen, 2006). After the glucose load, all the four groups exhibited the same glucose tolerance pattern where it increased significantly after the glucose load and eventually decreased to near fasting levels over the next 30 minutes, except for leptin only treated group ( $M = 9.18$ ,  $SEM = 0.65$ ) where there was a slight delay in glucose clearance starting at t15. The difference was statistically significant when compared to leptin-exercise group ( $M = 6.62$ ,  $SEM = 0.19$ ) at t30. It was nevertheless also higher than those in control and exercise groups ( $M = 6.90$ ,  $SEM = 0.55$  and  $M = 7.03$ ,  $SEM = 0.94$  respectively). A somewhat similar pattern of glucose peak following glucose loading has also been observed in both rat and mice respectively (Harris, 1998; Singh & Garland, 1989) despite the different routes of glucose loading (intravenous and oral glucose administration). Although the peak serum glucose level was slightly higher in the oral glucose loading (Harris, 1998), the difference may relate to different routes (oral) of glucose administration, the amount of glucose given (50 mg in mice), and the species of animal used. Although studies have shown that leptin enhances whole body glucose utilization in rats and mice (Ceddia et al., 1998; Houseknecht & Portocarrero, 1998; Park & Ahima, 2014; Fernández-Formoso et al., 2015), this however did not appear to be the case in this study. Leptin treated rats appeared to show a slight delay in the return of blood glucose level to the fasting levels indicating that 42 days of single dose of leptin treatment might reduce insulin action (Table 2). Reports on this in the literature are somewhat conflicting. The observations in the present study are in contrast to those reported in the mice following a 7-days infusion of leptin to lean mice. Lean mice treated with 10 µg/day leptin infusion for 7 days, for example, showed an almost normal glucose clearance after the glucose challenge (Harris, 1998). Studies in human with T2DM on leptin replacement therapy also showed normalization of glucose levels (Paz-Filho, Mastronardi, & Licinio, 2014). In contrast, two-hour glucose levels after a 75 g glucose tolerance test in non-diabetic women showed that leptin was associated with insulin resistance (Lee et al., 2009). The effect of leptin on tissue glucose utilization is closely related to the effect of leptin on insulin responsiveness. Leptin at a dose of 100 nM increases glucose uptake, lactate formation and glycogen synthesis and also potentiated the effect of physiological insulin concentration of glycogen synthesis in muscle (Ceddia et al., 1998). Leptin exerts an insulin-like effect in the skeletal muscle of lean rats. It was found that leptin stimulated glucose transport via PI3K pathway (Donato Jr, Frazão, & Elias, 2010) as there was a significant reduction (about 40%) on glucose transport when PI3K inhibitor (Wortmannin) was used (Berti & Gammeltoft, 1999). This suggested a crosstalk between leptin and insulin intracellular signaling pathways at the level of PI3K. It is known that the effect of insulin on glucose uptake in skeletal muscle is mainly mediated by GLUT4 translocation. Leptin has also been shown to increase PI3K activity by three fold (Berti, Kellerer, Capp, & Haring, 1997). Although many studies seem to suggest that leptin might stimulate glucose clearance, the reason for the contrasting results in this study is unclear. One possible reason could be the duration of leptin treatment. Most studies have either given leptin as a single dose or as a continuous intravenous dose over a period of 7-10 days. Perhaps prolonged exposure to leptin could lead to altered glucose clearance response. It is possible that chronic hyperleptinemia could decrease glucose uptake by the muscle. Studies found that hyperleptinemia in T2DM patients was also associated with increased TRB3 protein expression and insulin response was reduced by TRB3 binding to Akt thus decreasing insulin-stimulated glucose clearance (Marinho, Mekary, Muñoz, Gomes, Pauli & de Moura, 2015; Liu et al., 2010). There might be a connection between leptin and TRB3 levels perhaps, in part, affecting glucose uptake in the muscle. It is possible that leptin might be up-regulating TRB3 expression and with it increasing insulin resistance.

It was interesting to note the delayed glucose clearance was absent in leptin-treated rats that were exercised (Table 2). Physical exercise decreases leptin concentration and thus could possibly reverse leptin effects on glucose clearance as exercise increases glucose uptake and insulin sensitivity (Friedrichsen, Mortensen, Pehmøller, Birk, & Wojtaszewski, 2012; Jimenez-Pavon et al., 2012).

Physical exercise promotes glucose uptake into the skeletal muscle and ameliorates insulin responsiveness in the working muscle (Röhling, Herder, Stemper & Müssig, 2016; Bradley et al., 2014). Exercise also increases glycogen synthase activity in the muscle (Jensen & Richter, 2012). A study on muscle specific insulin receptor knockout mice showed exercise could normally increase muscle glucose transport even in the absence or near absence of muscle insulin receptor (Wojtaszewski et al., 1999). It is hypothesized that exercise induces re-localization of insulin's intracellular signaling molecules such as PI3K, Akt and GSK3 causing them to be more accessible for activation (Röhling, Herder, Stemper & Müssig, 2016; Wojtaszewski et al., 1999), and that it increases the downstream phosphorylation of TBC1D4, which can last many hours post exercise (Jensen & Richter, 2012). These could enhance responsiveness for activation by insulin and also glucose uptake into the skeletal muscle (Röhling, Herder, Stemper & Müssig, 2016; Wojtaszewski et al., 1999; Douen et al., 1990). The leptin-only group had reduced glucose clearance as the effect of leptin, and given that exercise increases glucose uptake, this could have been neutralized or negated the leptin effect on glucose uptake. This explains the normal glucose clearance in leptin-exercise group when challenged with exogenous glucose.

### **Serum insulin levels**

Fasting serum insulin levels were not different between the groups and a significant increase in serum insulin at five minutes after the glucose load was observed in all the groups (not shown in Table 3). Insulin levels normally increase following glucose loading, be it oral or intravenous. A similar insulin response to glucose challenge has also been reported before (Figueira, Ribeiro, Ignacio-Sauza, Vercesi, Carneiro & Oliveira, 2012; Harris, 1998). The insulin concentrations in mice that were treated with leptin and challenged with glucose show a same pattern (no different in fasting insulin levels and significant increase after glucose load) with comparable values to the findings in this study (Harris, 1998). Insulin levels were slightly lower at all times after the glucose load in all the experimental or treated groups when compared to those in the control rats, particularly in the exercised groups. The insulin level at five minutes after the glucose challenge was significantly lower in exercise ( $M = 2.61$ ,  $SEM = 0.49$ ), and leptin and exercise ( $M = 2.19$ ,  $SEM = 0.37$ ) treated rats ( $P < 0.05$  and  $P < 0.01$  respectively) when compared to control ( $M = 4.77$ ,  $SEM = 0.67$ ) while leptin group ( $M = 2.94$ ,  $SEM = 0.58$ ) was not significantly differs from either groups at t5. The precise reason for this is unclear. It is difficult to say if the slightly lower insulin response is due to the leptin or the exercise but as it is somewhat more evident in the exercise groups it might be due to the effect of exercise. Blunted insulin response to glucose challenge with significant hyperglycaemia following exercise has been reported before in humans (Chacko, 2016; Glidden, 2010; Heath et al., 1983). This phenomenon has been ascribed to long-term adaptations to training, reduced adiposity and perhaps also to the residual effects of the last bout of exercise. Rats in this study were exercised for 6 weeks and glucose challenge was performed 24 hours after the last bout of exercise. It is therefore possible that the slightly lower insulin response in the exercised rats might be due to the effect of exercise *per se*. The exact mechanism for this blunted insulin response remains unknown. Of interest to note is that despite the slightly lower insulin level, glucose levels between the control and exercised groups were not different, suggesting possibly increased insulin action in exercised rats.

### **Insulin action**

In this study, insulin action seems to improve with exercise as evident from the insulin levels after the glucose challenge when compared to those in the controls (Table 3). Despite the lower insulin levels, reports have shown that physical activity or exercise enhances glucose transport in muscle and this mechanism involves an insulin-independent muscle glucose transport (Messina et al., 2015; Lund, Holmant, Schmitz, & Pedersen, 1995). Contraction-stimulated glucose transport response is associated with metabolic feedback signals via AMPK and mechanical stress-activated signals (Jensen et al., 2014). Moreover, the enhanced glucose transport after exercise is due to translocation of GLUT4 to the cell surface that is different from that of the insulin-stimulated GLUT4 pool

(Messina et al., 2015; Douen et al., 1990). These reports support the results in this study that show normal glucose clearance despite a significantly lower insulin level in exercised rats ( $P < 0.05$  and  $P < 0.01$  in exercise and leptin-exercise groups respectively). Leptin treatment and exercise improved insulin action and the effects are additive. Increased peripheral blood flow during exercise amplifies total insulin delivery to the contracted muscle and thus compensates at least in part for the lessening insulin concentration during the glucose challenge. Furthermore, it appears conceivable that muscle might interact with pancreas and modulate insulin secretion for appropriate peripheral intracellular glucose disposal via interleukin-6 (IL-6), which is a most possible muscle-derived candidate protein for auto/para/endocrine action (Mizgier, Casas, Contreras-Ferrat, Llanos, & Galgani, 2014). It is well documented that acute and transient inflammation due to exercise can increase muscle-derived plasma IL-6 even after a single bout of exercise (Hennigar, McClung & Pasiakos, 2017; Ostrowski, Rohde, Asp, Schjerling, & Pedersen, 1999). A study on C2C12 myotubes found that both protein and mRNA expression of IL-6 were significantly up regulated in contracting myotubes during a 24-hours period (Farmawati et al., 2013). Moreover, this contractile activity improved their response to insulin in terms of glycogen accumulations. Thus we speculate that six-weeks of exercise might decrease the demand for insulin for glucose uptake by the skeletal muscle and this might be mediated by IL-6 and that improvement in insulin action with exercise is additive to leptin treatment.

## **CONCLUSION**

The overall aim of this study was to investigate the effects of chronic leptin treatment and exercise on body weight, serum glucose and insulin levels after a glucose challenge. To address this issue, rats were treated with exogenous leptin and were also exercised for 42 days (6 weeks) followed by a glucose challenge. Despite no significant differences in body weight between the groups, leptin group showed a slightly higher trend of mean body weight compared to other groups. Results from the glucose challenge provide evidence of contrasting effects of chronic leptin treatment and exercise on glucose clearance and insulin levels and function. Serum insulin levels following a glucose challenge were somewhat lower in leptin and exercised rats when compared to the response in the controls. Despite this, the rats were still able to maintain normal glucose clearance by increasing insulin action. Exercise also reduced insulin levels but increased insulin responsiveness. Exercise reduced insulin levels after glucose load yet preserving normal glucose clearance by significantly increased insulin action.

Based on the results, it can be concluded that chronic administration of leptin for 42 days induced insulin resistance. Late leptin effect was noted by suppressed glucose clearance. This chronic leptin administration was designed to mimic hyperleptinemia in obese individuals and findings in this study suggest that hyperleptinemia decreases insulin secretion and action. On the other hand, exercise reverses the leptin effects by promoting glucose clearance via insulin-independent pathway and significantly increasing insulin action. Collectively, the studies presented in this study highlight the effect of leptin and exercise in biological action on glucose homeostasis.

## **RECOMMENDATION**

For future studies, exploring the insulin-signaling mechanism by leptin treatment with insulin load in addition to glucose tolerance test to mimic hyperinsulinemia in type 2 diabetic patients would also add more information to the investigation.

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## REFERENCES

- Berti, L., & Gammeltoft, S. (1999). Leptin stimulates glucose uptake in C2C12 muscle cells by activation of ERK2. *Molecular and Cellular Endocrinology*, 157, 121–130.
- Berti, L., Kellerer, M., Capp, E., & Haring, H. U. (1997). Leptin stimulates glucose transport and glycogen synthesis in C2C12 myotubes: evidence for a PI 3-kinase mediated effect. *Diabetologia*, 40(5), 606–609.
- Bird, S. R., & Hawley, J. A. (2017). Update on the effects of physical activity on insulin sensitivity in humans. *BJM Open Sport Exerc Med*, 2(1), e000142.
- Blüher, M., & Mantzoros, C. S. (2015). From leptin to other adipokines in health and disease: facts and expectations at the beginning of the 21<sup>st</sup> century. *Metabolism*, 64(1), 131-145.
- Blüher, M., Michael, M. D., Peroni, O. D., Ueki, K., Carter, N., Kahn, B. B., & Kahn, C. R. (2002). Adipose tissue selective insulin receptor knockout protects against obesity and obesity-related glucose intolerance. *Developmental Cell*, 3(1), 25–38.
- Bouassida, A., Chamari, K., Zaouali, M., Feki, Y., Zbidi, A., & Tabka, Z. (2010). Review on leptin and adiponectin responses and adaptations to acute and chronic exercise. *Br J Sports Med*, 44, 620–630.
- Bouassida, A., Zalleg, D., Zaouali, M., Gharbi, N., Fekih, Y., Richalet, J. P., & Tabka, Z. (2004). Effects of supra-maximal exercise on plasma concentrations of leptin. *Science & Sports*, 19, 136-138.
- Bradley, H., Shaw, C. S., Worthington, P. L., Shepherd, S. O., Cocks, M., & Wagenmakers, A. J. M. (2014). Quantitative immunofluorescence microscopy of subcellular GLUT4 distribution in human skeletal muscle: effects of endurance and sprint interval training. *Physiological Reports*, 2(7), 1–16.
- Brüning, J. C., Michael, M. D., Winnay, J. N., Hayashi, T., Hörsch, D., Accili, D., Goodyear, L. J., & Kahn, C. R. (1998). A muscle-specific insulin receptor knockout exhibits features of the metabolic syndrome of NIDDM without altering glucose tolerance. *Molecular Cell*, 2(5), 559–569.
- Burnett, L. C., Skowronski, A. A., Rausch, R., LeDuc, C. A., & Leibel, R. L. (2017). Determination of the half-life of circulating leptin in the mouse. *International Journal of Obesity*, 41(3), 355–359.
- Cantley, J. (2014). The control of insulin secretion by adipokines: current evidence for adipocyte-beta cell endocrine signalling in metabolic homeostasis. *Mamm Genome*, 25, 442–454.
- Ceddia, R. B., William Jr, W. N., & Curi, R. (1998). Leptin increases glucose transport and utilization in skeletal muscle in vitro. *Gen. Pharmac*, 31(5), 799–801.
- Chacko E. (2016). Exercising tactically for taming postmeal glucose surges. *Scientifica*, 2016, 4045717.
- Chen, C., Chen, H. J., Qian, L. H., Jiang, M. H., & Chen, G. Y. (2006). Effect of different blood glucose levels on the expression of cerebral GLUT3 mRNA in neonatal rats with hypoxia-ischemia. *Chinese Journal of Contemporary Pediatrics*, 8(5), 395–401.
- Cignarelli, A., Genchi, V. A., Perrini, S., Natalicchio, A., Laviola, L., & Giorgino, F. (2019). Insulin and insulin receptors in adipose tissue development. *International journal of molecular sciences*, 20(3), 759.
- David, B. W., John, P. H. W. (2014). Chapter 15 - Glucose metabolism and the pathophysiology of diabetes mellitus. *Clinical Biochemistry: Metabolic and Clinical Aspects (Third Edition)*. 273-304. Churchill Livingstone
- De Almeida, R. B., Pauli, L. S., de Souza, C. T., da Silva, A. S., Cintra, D. E., Marinho, R., De Moura, L. P., Ropelle, E. C., & Pauli, J.R. (2014). Acute exercise decreases TRB3 protein levels in the hypothalamus of obese rats. *Med Sci Sports Exerc*.
- De Meyts, P. (2016). The insulin receptor and its signal transduction network. In: Feingold, K. R., Anawalt, B., Boyce, A., South Dartmouth: MDText.com, Inc.; 2000-. Available from <https://www.ncbi.nlm.nih.gov/books/NBK378978/>
- Donato Jr., J, Frazão, R. & Elias, C. F. (2010). The PI3K signaling pathway mediates the biological effects of leptin. *Arquivos Brasileiros de Endocrinologia & Metabologia*, 54(7), 591-602.
- Douen, A. G., Ramlal, T., Rastogi, S., Bilan, P. J., Cartee, G. D., Vranic, M., Holloszy, J. O., & Klip, A. (1990). Exercise induces recruitment of the "insulin-responsive glucose transporter". *The Journal of Biological Chemistry*, 265(23), 13427–13430.
- Ekmen, N., Helvacı, A., Gunaldi, M., Sasani, H., & Yildirmak, S. T. (2016). Leptin as an important link between obesity and cardiovascular risk factors in men with acute myocardial infarction. *Indian Heart Journal*, 68(2), 132–137.
- Farmawati, A., Kitajima, Y., Nedachi, T., Sato, M., Kanzaki, M., & Nagatomi, R. (2013). Characterization of contraction-induced IL-6 up-regulation using contractile C2C12 myotubes. *Endocrine Journal*, 60(2), 137–147.

- Fernández-Formoso, G., Pérez-Sieira, S., González-Touceda, D., Dieguez, C., & Tovar, S. (2015). Leptin, 20 years of searching for glucose homeostasis. *Life Sciences*.
- Figueira, T. R., Ribeiro, R. A., Ignacio-Sauza, L. M., Vercesi, A. E., Carneiro, E. M., & Oliveira, H. C. F. (2012). Enhanced insulin secretion and glucose tolerance in rats exhibiting low plasma free fatty acid levels and hypertriglyceridaemia due to congenital albumin deficiency. *Exp Physiol*, 97(4), 525-533
- Friedrichsen, M., Mortensen, B., Pehmøller, C., Birk, J. B., & Wojtaszewski, J. F. P. (2012). Exercise-induced AMPK activity in skeletal muscle: Role in glucose uptake and insulin sensitivity. *Molecular and Cellular Endocrinology*, 1–11.
- Glidden, A. R. (2010). *The blunted insulin release after exercise and the relationship with gastric inhibitory polypeptide and glucagon-like peptide-1*. Iowa State University.
- Haron, M. N., D'Souza, U. J. A., Jaafar, H., Zakaria, R., & Singh, H. J. (2010). Exogenous leptin administration decreases sperm count and increases the fraction of abnormal sperm in adult rats. *Fertility and Sterility*, 93(1), 322-324.
- Harris, R. B. S. (1998). Acute and chronic effects of leptin on glucose utilization in lean mice. *Biochemical and Biophysical Research Communications*, 245(2), 502–509.
- Heath, G. W., Gavin, J. R., Hinderliter, J. M., Hagberg, J. M., Bloomfield, S. A., & Holloszy, J. O. (1983). Effects of exercise and lack of exercise on glucose tolerance and insulin sensitivity. *J Appl Physiol Respir Environ Exerc Physiol*, 55(2), 512–517.
- Hennigar, S. R., McClung, J. P., & Pasiakos, S. M. (2017). Nutritional interventions and the IL-6 response to exercise. *The FASEB Journal*, 31, 3719-3728.
- Houseknecht, K. L., & Portocarrero, C. P. (1998). Leptin and its receptors : regulators of whole-body energy homeostasis. *Domest Anim Endocrinol*, 15(6), 457–475.
- Ibrahim, K. S., Omar, E., Ruth, G., Froemming, A., & Singh, H. J. (2013). Leptin increases blood pressure and markers of endothelial activation during pregnancy in rats. *BioMed Research International*, 2013.
- Ishii, T., Yamakita, T., Yamagami, K., Yamamoto, T., Miyamoto, M., Kawasaki, K., Hosoi, M., Yoshioka, K., Sato, T., Tanaka, S., & Fujii, S. (2001). Effect of exercise training on serum leptin levels in type 2 diabetic patients. *Metabolism*, 50(10), 1136–1140.
- Jensen, T. E., & Richter, E. A. (2012). Regulation of glucose and glycogen metabolism during and after exercise. *J Physiol*, 590.5(March), 1069–1076.
- Jensen, T. E., Sylow, L., Rose, A. J., Madsen, A. B., Angin, Y., Maarbjerg, S. J., & Richter, E. A. (2014). Contraction-stimulated glucose transport in muscle is controlled by AMPK and mechanical stress but not sarcoplasmic reticulum Ca<sup>2+</sup> release. *Molecular Metabolism*, 3(7), 742–753.
- Jimenez-Pavon, D., Ortega, F. B., Artero, E. G., Labayen, I., Vicente-Rodriguez, G., Huybrechts, I., Moreno, L. A., Sjostrom, M., Castillo, M. J., Gonzalez-Gross, M., & Ruiz, J. R. (2012). Physical activity, fitness, and serum leptin concentrations in adolescents. *The Journal of Pediatrics*, 160(4), 598-603.
- Joseph, J. W., Koshkin, V., Saleh, M. C., Sivitz, W. I., Zhang, C.-Y., Lowell, B. B., ... Wheeler, M. B. (2004). Free Fatty Acid-induced  $\beta$ -Cell Defects Are Dependent on Uncoupling Protein 2 Expression. *Journal of Biological Chemistry*, 279(49), 51049–51056.
- Kang, S., Kim, K. B., & Shin, K. O. (2013). Exercise training improve leptin sensitivity in peripheral tissue of obese rats. *Biochemical and Biophysical Research Communications*, 435(3), 454-459.
- Katherine, A. T., Alexandra, J. M. & Jessica, H. B. (2019). Chapter 12 - Influence of hormone on the development of eating disorder. Eating disorders and obesity in children and adolescents. *Elsevier*. 73-77. ISBN 9780323548526.
- Kumar, M. V., Shimokawa, T., Nagy, T. R., & Lane, M. D. (2002). Differential effects of a centrally acting fatty acid synthase inhibitor in lean and obese mice. *Proceedings of the National Academy of Sciences of the United States of America*, 99(4), 1921–1925.
- Lalanza, J. F., Sanchez-Roige, S., Cigarroa, I., Gagliano, H., Fuentes, S., Armario, A., Capdevila, L., & Escorihuela, R. M. (2015). Long-term moderate treadmill exercise promotes stress-coping strategies in male and female rats. *Scientific Reports*, 5:16166.
- Lee, C., Lee, C., Tsai, S., Huang, C., Wu, M., Tai, S.-Y., Lin, F.-F., Chao, N.-C., & Chang, C.-J. (2009). Association between serum leptin and adiponectin levels with risk of insulin resistance and impaired glucose tolerance in non-diabetic women. *The Kaohsiung Journal of Medical Sciences*, 25(3), 116–125.
- Leto, D., & Saltiel, A. R. (2012). Regulation of glucose transport by insulin: traffic control of GLUT4. *Nature Reviews Molecular Cell Biology*, 13(6), 383-396.
- Liu, J., Wu, X., Franklin, J. L., Messina, J. L., Hill, H. S., Moellering, D. R., Walton, R. G., Martin, M., & Garvey, W. T. (2010). Mammalian Tribbles homolog 3 impairs insulin action in skeletal muscle: role in glucose-induced insulin resistance. *Am J Physiol Endocrinol Metab*, 298, E565–E576.

- Lund, S., Holmang, G. D., Schmitz, O., & Pedersen, O. (1995). Contraction stimulates translocation of glucose transporter GLUT4 in skeletal muscle through a mechanism distinct from that of insulin. *Proceedings of the National Academy of Sciences of the United States of America*, 92(13), 5817–5821.
- Marinho, R., Mekary, R. A., Muñoz, V. R., Gomes, R. J., Pauli, J. R., & de Moura, L. P. (2015). Regulation of hepatic TRB3/Akt interaction induced by physical exercise and its effect on the hepatic glucose production in an insulin resistance state. *Diabetology & Metabolic Syndrome*, 7, 67.
- Melmer, A., Kempf, P., Laimer, M. (2018). The role of physical exercise in obesity and diabetes. *Praxis*, 107, 971-976.
- Messina, G., Palmieri, F., Monda, V., Messina, A., Dalia, C., Viggiano, A., Tafuri, D., Moscatelli, F., Valenzano, A., Cibelli, G., Chieffi, S., & Monda, M. (2015). Exercise causes muscle GLUT4 translocation in an insulin-independent manner. *Biol Med (Aligarh)*, 1:006.
- Mizgier, M. L., Casas, M., Contreras-Ferrat, A., Llanos, P., & Galgani, J. E. (2014). Potential role of skeletal muscle glucose metabolism on the regulation of insulin secretion. *Obesity Reviews*, 15(7), 587–597.
- Nuri, R., Moghaddasi, M., Darvishi, H., Izadpanah, A. (2016). Effect of aerobic exercise on leptin and ghrelin in patients with colorectal cancer. *Journal of Cancer Research and Therapeutics*, 12(1), 169-174.
- Ostrowski, K., Rohde, T., Asp, S., Schjerling, P., & Pedersen, B. K. (1999). Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans. *Journal of Physiology*, 515(1), 287–291.
- Park, H.-K., & Ahima, R. S. (2014). Physiology of leptin: energy homeostasis, neuroendocrine function and metabolism. *Metabolism*, 1–11.
- Paz-Filho, G., Mastroradi, C. A., & Licinio, J. (2014). Leptin treatment: Facts and expectations. *Metabolism*.
- Röder, P. V., Wu, B., Liu, Y., & Han, W. (2016). Pancreatic regulation of glucose homeostasis. *Experimental & Molecular Medicine*, 48(3), e219.
- Röhling, M., Herder, C., Stemper, T., & Müssig, K. (2016). Influence of acute and chronic exercise on glucose uptake. *J Diabetes Res*, 1-33.
- Sáinz, N., Barrenetxe, J., Moreno-Aliaga, M. J., & Martínez, J. A. (2015). Leptin resistance and diet-induced obesity: central and peripheral actions of leptin. *Metabolism*, 64(1), 35-46.
- Sari, R., Balci, M. K., & Apaydin, C. (2010). The relationship between plasma leptin levels and chronic complication in patients with type 2 diabetes mellitus. *Metabolic Syndrome and Related Disorder*, 8(6), 499-503.
- Sasaki, H., Ohtsu, T., Ikeda, Y., Tsubosaka, M., & Shibata, S. (2014). Combination of meal and exercise timing with a high-fat diet influences energy expenditure and obesity in mice. *Chronobiology International*, 1–17.
- Singh, H. J., & Garland, H. O. (1989). A comparison of the effects of oral and intravenous glucose administration on renal calcium excretion in the rat. *Quarterly Journal of Experimental Physiology*, 74, 531–540.
- Sjøberg, K. A., Frøsig, C., Kjøbsted, R., Sylow, L., Kleinert, M., Betik, A. C., Shaw, C. S., Kiens, B., Wojtaszewski, J. F. P., Rattigan, S., Richter, E. A. and McConell, G. K. (2017). Exercise increases human skeletal muscle insulin sensitivity via coordinated increases in microvascular perfusion and molecular signaling. *Diabetes*, 66(6), 1501-1510.
- Way, K. L., Hackett, D. A., Baker, M. K., & Johnson, N. A. (2016). The effect of regular exercise on insulin sensitivity in type 2 diabetes mellitus: A systematic review and meta-analysis. *Diabetes & Metabolism Journal*, 40(4), 253–271.
- Wojtaszewski, J. F. P., Higaki, Y., Hirshman, M. F., Michael, M. D., Dufresne, S. D., Kahn, C. R., & Goodyear, L. J. (1999). Exercise modulates postreceptor insulin signaling and glucose transport in muscle-specific insulin receptor knockout mice. *The Journal of Clinical Investigation*, 104(9), 1257–1264.
- Zeng, J., Patterson, B. W., Klein, S., Martin, D. R., Dagogo-Jack, S., Kohrt, W. M., Miller, S. B., & Landt, M. (1997). Whole body leptin kinetics and renal metabolism in vivo. *The American Journal of Physiology*, 273(6 Pt 1), E1102–E1106.

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