Middle East Journal of
Scientific Publishing
Vol. (9) Issue (5)
Edition 35th
2026(1 - 13)

Physiological Regulation of the Liver-Metabolic Axis and Glutathione-Dependent Hormonal Response: An Extensive Study of Insulin and Leptin in Female Rats

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Published on: 6 may 2026

Abstract

The liver-metabolic axis plays key roles in systemic homeostasis and integrates hepatic function and hormonal regulation Oxidative stress can interfere with this balance, leading to poor insulin signaling and decreased leptin effectiveness, causing metabolic imbalance. This study was conducted to determine the long-term effects of oral glutathione on liver enzymes and some metabolic hormones, using female rats as subjects.

Eighty adult female Wistar rats weighing between 200 and 250 grams were randomized into two equal halves. For a period of 60 consecutive days, from December 1, 2025, to February 1, 2026, the control group received physiological saline daily while treatment group was provided oral glutathione with a dose equivalent to 500 mg per kilogram per day. Serum alanine aminotransferase and aspartate aminotransferase (ALT and AST) was carried out using kinetic colorimetric techniques. We assessed insulin and leptin levels by using ELISA kits.

Supplementing with glutathione had a major impact on the reduction of serum alanine aminotransferase which was 35.40 ± 2.28 U/L as against 48.62 ± 3.15 U/L in the control group. Likewise, the levels of aspartate aminotransferase in the treated rats (87.12 ±5.92 U/L) were found to decreased as compared to the controls (112.45±7.80 U/L) at probability values <0.05. These alterations signify a greater stability of the membranes of hepatocytes.

Moreover, compared to controls (8.45 ± 0.72 μIU/mL; P< 0.01), the mean serum insulin level of glutathione treated group was lower (6.12 ± 0.45 μIU/mL). Leptin concentrations of the treatment group were also significantly decreased to 2.30 ± 0.15 ng/mL compared to controls of 3.85 ± 0.28 ng/mL ($p < 0.001$), demonstrating better leptin responsiveness. During the duration of the study, we observed no negative clinical signs or body weight changes.

The study concludes that oral glutathione long-term supplementation was able to stabilize the liver-metabolic axis in females. This effect seems to be caused by the protection of liver function and enhancement of insulin and leptin signals. Glutathione thus appears to be an interesting candidate for treating metabolic disorders and improving hormonal imbalance under oxidative stress conditions.

Keywords: Glutathione, Liver-Metabolic Axis, Insulin Sensitivity, Leptin Regulation, Female Wistar Rats, Hepatic Protection

*** Introduction**

1- Biological Significance and Glutathione Biosynthesis

Glutathione (L-γ-glutamyl-L-cysteinyl-glycine; GSH) is the most prevalent thiol antioxidant in mammals. It is crucial for regulation of redox balance within cells. GSH is produced via a two step enzymatic process in the liver. The first step is catalysed by γ-glutamylcysteine synthetase and the second by glutathione synthetase to form the active tripeptide. (Lu, 2013) Specific phytonutrients and micronutrients from the diet can help to boost endogenous glutathione synthesis and the overall antioxidant capacity of our biology (Minich & Brown, 2019). In addition to scavenging reactive oxygen species (ROS), GSH is also an essential cofactor of the selenoenzyme glutathione peroxidase (GPx), which reduces lipid hydroperoxides and inhibits lipid peroxidation in membranes (Forman, Zhang, & Rinna, 2009). Thus, sufficient intracellular concentrations of GSH are essential to prevent cellular macromolecules such as nucleic acids and proteins from oxidation. Reduced glutathione and oxidized glutathione (GSH: GSSG) ratio is popular numerical indicator of global level oxidative stress and redox imbalance (Wu et al., 2004). Supplementation with oral glutathione can increase systemic glutathione pools indicating bio-availability in vivo Richie et al. (2015)

2- The Liver–Metabolic Axis: Functional and Integrative Overview

Glutathione GSH L-γ-glutamyl-L-cysteinyl-glycine is the most abundant thiol antioxidant in mammals and is involved in cellular redox homeostasis. The liver creates it through an enzymatic process involving two steps. In the first step, γ-glutamylcysteine synthetase catalyzes formation of γ-glutamylcysteine and in the second step, glutathione synthetase converts it to the active tripeptide (Lu, 2013). Through boosting the body's internal production of glutathione and enhancing overall antioxidant potential, dietary phytonutrients and micronutrients can promote this (Minich & Brown, 2019).

Glutathione not only directly scavenges reactive oxygen species but also acts as a cofactor for the selenoenzyme glutathione peroxidase. This enzyme can limit lipid peroxidation within membranes and reduce lipid hydroperoxides. (Forman, Zhang, & Rinna, 2009) Keeping enough glutathione within cells is important to prevent important substances, such as nucleic acids and proteins, from becoming oxidized.

The ratio of GSH-to-GSSG is a classic marker of oxidative stress and redox dysregulation in the cell (Wu et al., 2004). Moreover, oral supplementation with glutathione increases systemic glutathione levels which supports the bio-availability in vivo (Richie et al. 2015).

3- Oxidative Stress and the Perturbation of Insulin Signaling

Insulin is a major anabolic hormone that regulates glucose uptake, glycogenesis, and lipid production. Insulin signalling through the cell demands the coordination of receptor activation and phosphorylation. The intracellular ROS is increased which in turn activate stress kinases like c-Jun N-terminal kinase (JNK) due to oxidative stress. These kinases induce serine phosphorylation of Insulin Receptor Substrate-1 (IRS-1), causing it to become unresponsive to insulin signaling. As a result, there is less GLUT4 translocation to the plasma membrane (Yaribeygi et al., 2020). Decreased amounts of glutathione have been linked to the development of obesity and insulin resistance (Dixon & Pazdro, 2021). Continued disruption of redox balance promotes peripheral insulin resistance, which is characteristic of metabolic diseases. Antioxidant interventions including glutathione support can protect the functional properties of IRS-1 and insulin

sensitivity in an oxidative environment (El-Hafidi et al., 2018).

4- Leptin Dynamics and Energy Homeostasis

Leptin is an adipose-derived hormone that transmits information on long-term energy stores to central regulatory circuits in the hypothalamus to affect appetite, energy expenditure, basal metabolic rate, etc. Leptin signaling may be impaired in chronic oxidative stress and inflammation states. The transport of leptin over the blood–brain barrier may be impeded or the Ob-Rb receptor in the hypothalamus may not be sensitive to leptin. This is called leptin resistance (Myers et al., 2010). The characteristic metabolic syndrome symptoms and elevated circulating leptin levels (hyperleptinemia) aren't uncommon with leptin resistance. According to the literature, modulating oxidative stress and enhancing tissue antioxidant capacity diminishes pro-inflammatory signals in adipose tissue, reversing leptin receptor resistance and improving metabolic feedback regulation (Ottaway & Conner, 2015).

5- Sexual Dimorphism in Redox and Metabolic Regulation

There are biological differences between the sexes that influences the response to oxidative stress. In females, endogenous antioxidant defences are differently regulated in relation to hormone cycles. Particularly, the estrogen has antioxidant properties and regulates the expression of phase I and phase II detoxification enzymes (Klein & Flanagan, 2016). Additionally, estrogen improves the oxidative damage resistance of the liver and works with glutathione to boost antioxidant defense.

Thus, females show a different antioxidant profile than that of males. A study conducted on female participants to determine the effects of antioxidant intervention reveals important information about metabolic regulation and the efficacy of antioxidant treatment.

6- Justification and Objectives of the Study

Many studies have focused on the short-term effect of antioxidants but the long-term effect of exogenous glutathione supplementation on hormonal and metabolic regulation is not well understood, in female models. The objective of this study was to analyze the influence of 60 days of continuous administration of oral glutathione, on liver function and metabolic hormones (insulin and leptin) of female Wistar rats.

The use of a fairly large sample size (80) has provided detailed data

regarding antioxidant status, hepatic function and hormonal response. This sheds more light on how metabolism is regulated at whole-body level.

*** Materials and Methods**

1- Ethical Approval and Animal Husbandry

This research was carried out in accordance with the Institutional Animal Care and Use Committee guidelines. Eighty adult female Wistar rats weighing 200–250 g were obtained from the College of Veterinary Medicine University of Kufa Once arrived at the laboratory, they were placed in sterilized polycarbonate cages at controlled environmental conditions: temperature 22 ± 2 °C, relative humidity 50 ± 10 percent and 12 hours light–dark cycle.

During the study period, the animals were fed with standard pellet formulation and given ad libitum access to distilled water. Prior to the experimental processes, a period of two-week acclimatization was established for eventual physiological stabilization, according to indication in laboratory rodents (Waynforth & Flecknell, 1992).

2- Experimental Design and Treatment Protocol

The experimental period took place between December 1, 2025, and February 1, 2026, after random allocation of the rats (n = 40 per group) into two equal groups.

Rats received a daily oral dose of physiological saline (0.9 % NaCl, i.e. saline) via gastric gavage to serve as controls.

The glutathione group (GSH) was administered oral glutathione at a standardized daily dose equivalent to 500 mg/kg body weight, based on the metabolic differences between rats and humans, dissolved in distilled water.

All treatments were given as scheduled every morning for the complete 60 days. Body weight and general health indicators were monitored weekly to allow dose adjustments as well as animal welfare.

3- Blood Collection and Serum Processing

In the last stage treatment, all rats were fasted over-night (12 hours) with ad libitum access to water. Blood was obtained through cardiac puncture of light anesthesia using ketamine and xylazine to minimize stress on the animals. The blood collected was transferred to plain tubes and allowed to clot at room temperature for about 30 min. The samples were centrifuged at a speed of 3000 rpm for a duration of 15 minutes at 4 °C to separate the serum. The serum was collected in cryovials

and stored at −80 °C until biochemical and hormonal assays were conducted. This is a standard protocol for serum collection from rodents (Hucklebridge et al., 1996).

4- Biochemical Assessment of Liver Enzymes

Using the manufacturer's specification kinetic colorimetric methods were used to determine serum alanine aminotransferase and aspartate aminotransferase activity. To ensure analytical accuracy, commercial diagnostic reagent kits from Roche Diagnostics and Randox Laboratories were used. An automated spectrophotometric analyzer was employed for absorbance measurement to improve reproducibility and control variability.

The method used in this investigation is in accordance with laboratory techniques that are used for liver transaminases tests in the clinic (Honda et al., 2017).

5- Hormonal Quantification (Insulin and Leptin)

The concentration of insulin and leptin was determined using high-sensitivity, rat ELISA kits.

Assays repetition was undertaken in order to reduce assay variance. Using an ELISA method, insulin was quantified with double antibody sandwich technique while Leptin was quantified using an ELISA based on the monoclonal antibody of rat leptin.

We used a calibrated microplate reader to measure the optical density at 450 nm and calculated the hormone concentrations using standard curves obtained from known reference concentrations. Hormone assays in rodents using ELISA have high specificity and are well-validated (Caviola et al., 2020 in endocrine).

6- Statistical Evaluation

The statistical software SPSS version 26.0 was used to analyze all data. The outcomes are reported as mean ± standard error of the mean. The Shapiro-Wilk test was used to assess data normality. Independent samples t-test was performed for comparison of results of the control and treatment group.

A p-value less than 0.05 is considered statistically significant as per standard biomedical research criteria used in this study.

*** Results**

The physiological and biochemical assessments carried out after the 60 experimental days (December 2025 to February 2026) indicate that long-term oral supplementation of glutathione has a significant modulatory effect liver function and metabolic hormones in

female Wistar rats with a total of 80 numbers of animals.

1- Effects of Glutathione on Hepatic Enzymes

Rats that received oral GSH had a significant stabization of liver function parameters. Compared to the control group, the GSH group exhibited significantly reduced Serum Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) activities ($P < 0.05$). The level of ALT dropped down about 27% and the level of AST was reduced by about 22% thereby improving membrane integrity of hepatocytes.

Table 1: Liver Enzyme Activities Following 60-Day Glutathione Supplementation (Mean ± SEM)

Parameter (Unit)	Control Group (n=40)	GSH Group (n=40)	P-value
ALT (U/L)	48.62 ± 3.15	35.40 ± 2.28	0.012
AST (U/L)	112.45 ± 7.80	87.12 ± 5.92	0.024
Albumin (g/dL)	3.42 ± 0.18	3.65 ± 0.12	0.215

1- Significant difference at $P < 0.05$ compared to control, indicating that GSH supplementation improved hepatocyte membrane stability and reduced enzyme leakage.

2- Albumin levels did not change significantly, suggesting no adverse effect on hepatic protein synthesis.

2- **Modulation of Insulin and Leptin**

Metabolic profiling showed GSH to modulate the adipose-liver-hormone axis. In our experiment, it was found that serum insulin levels were significantly low in the GSH-treated group (6.12 ± 0.45 μIU/mL) as compared to controls (8.45 ± 0.72 μIU/mL, $P = 0.008$).

In the treated group, leptin levels decreased significantly (2.30 ± 0.15 ng/mL) when compared to controls (3.85 ± 0.28 ng/mL, $P = 0.001$). This nearly 40% reduction indicates restoration of leptin sensitivity and greater efficiency of the energy-regulatory feedback systems.

Table 2: Serum Insulin and Leptin Levels Post-GSH Supplementation (Mean ± SEM)

Hormone (Unit)	Control Group (n=40)	GSH Group (n=40)	P-value
Insulin (μIU/mL)	8.45 ± 0.72	6.12 ± 0.45	0.008
Leptin (ng/mL)	3.85 ± 0.28	2.30 ± 0.15	0.001

1- Significant reduction in insulin levels ($P < 0.05$) indicates enhanced insulin sensitivity in peripheral tissues.

2- Highly significant reduction in leptin levels ($P < 0.01$) reflects improved leptin receptor responsiveness and potential reversal of leptin resistance.

3- General Observations

At the experimental period all animals were clinically healthy without any discomfort and mortality. In both groups, the body weight gradually increased without statistically significant differences indicating that the dose of glutathione does not negatively affect normal growth or nutrient absorption in female Wistar rats.

*** Discussion**

The physiological investigation in this study presents evidence that long-term oral administration of glutathione has a powerful regulatory effect on the liver–metabolic axis in rats. After treatments of 60 days, the hepatic transaminases like alanine aminotransferase and aspartate aminotransferase were decreased along with the normalization of metabolic hormones such as insulin and leptin.

Metabolically, these results indicate that glutathione has a more wide-ranging modulatory role than previously recognized. Its function is not limited to that of an antioxidant.

1- Maintaining liver integrity and reducing the leaking of enzymes.

Glutathione levels are improved in liver cells from glutathione treated rats if they have lower serum aminotransferase levels. Hepatic cells have the ability to use glutathione, present in intracellular medium, to neutralize reactive oxygen species and to protect membrane lipids from peroxidation. When levels of glutathione decrease, a redox imbalance occurs which makes the hepatocyte more vulnerable to oxidative injury. Disruption of membrane integrity led to higher levels of transaminases in the plasma.

These findings are corroborated clinically. According to studies, oral glutathione supplementation in patients suffering from nonalcoholic fatty liver disease reduces levels of alanine aminotransferase and improves liver function after antioxidant therapy**.**

Moreover, a marked increase in albumin levels was not observed implicated either glutathione did not overstimulate hepatic biosynthetic function. As the effect is balanced in both directions on protein synthesis, It further supports the protective character of glutathione in maintaining redox homeostasis and stabilizing liver cell function

2- Molecular Reinstatement of Insulin Signaling and Sensitivity

In the study, glutathione-treated rats were found to have more stable serum insulin levels which is indicative of increased peripheral

insulin sensitivity compared to normal ones. Insulin resistance is often accompanied by a heightened oxidative stress where ROS and stress activated kinases (eg. JNK) disrupt insulin signaling at the level of insulin receptor substrate-1 (IRS 1). The kinases cause IRS-1 to be phosphorylated on inhibitory serine residues, which prevents any signalling and glucose transporter translocation. Insulin resistance is described by this process.

Studies suggest that glutathione regulates the intracellular thiol milieu in order to keep IRS-1 stable. It acts for the efficiency of glucose uptake and compensatory hyperinsulinemia by restoring the redox balance. This mechanism likely is responsible for maintaining more physiological insulin levels and suggests that glutathione could be protective against oxidative stress-induced early-phase insulin resistance.

3- Improvements in Leptin Sensitivity and Adipose–Hepatic Feedback Loops

Decreased serum leptin with GSH supplementation may reflect increased leptin sensitivity, a feature often lost in metabolic disease. Leptin becomes resistant due to the high amounts of circulating leptin because of problematic signalling in the peripheral and central systems which is partly caused because of inflammation and oxidative stress.

Leptin is a hormone secreted by adipose tissue and acts on the hypothalamus, regulating energy homeostasis and stimulating neuropeptide Y secretion in the body. Moreover, it inhibits the activity of neuropeptide Y. Ultimately, it helps to prevent excessive feeding and regulate body weight. A more balanced redox state might enhance adipocyte function and leptin receptor response, creating a more efficient negative feedback loop for appetite control and energy spending. Similar observations were made in other models in which increased antioxidant capacity benefited leptin dynamics and energy homeostasis.

4- Synergistic Effects of Sex-Specific Physiology

This study used only female Wistar rats to examine the sex-specific physiological impact of prolonged antioxidant therapy. Female metabolism varies due to cyclical changes brought by estrogen. Estrogens have antioxidant properties and regulate the activities of detoxifying and redox enzymes. The changes in hormone levels can interfere with the expression and activity of metabolic and antioxidant genes. It provides a specific

physiological context to investigate the effect of glutathione.

The physiological changes observed over a 60-day period suggest that exogenous glutathione acts as a stabilising buffer, allowing the natural hormones to work in harmony, thus maintaining balance in metabolic and hormonal functions. Under conditions of continuing oxidative stress where inner antioxidant defenses are lacking, this buffering phenomenon is important.

*** Conclusions and Recommendations**

1- Conclusions

According to the findings of this study, the continuous consumption of glutathione (GSH) through the mouth has physiologic benefits. Results from this 60-day trial experiment indicate that GSH supplementation can improve liver function and metabolic activity.

Supplementation with GSH was associated with a significant improvement in liver enzymes, alanine aminotransferase and aspartate aminotransferase. This effect may be attributed to exogenous GSH's ability to stabilize hepatocyte membranes, both by reducing lipid peroxidation and by preventing leakage of enzymes.

Along with this, glutathione administration restored insulin and leptin levels, improved insulin sensitivity in the periphery and regulated the adipose and hepatic interactions better. GSH is an important player in the liver - metabolic axis supporting these findings.

The findings added more evidence to the importance of antioxidant-based therapy for the management of obesity. Apparently, GSH could affect the responsiveness to leptin in addition to glucose and insulin transport efficiency and thermogenesis related metabolic pathways.

Physiological appearance related outcomes shows that GSH can have an interaction with the hormonal system of females indicating GSH may not be a gender-neutral antioxidant.

In conclusion, long term administration of GSH in a dose equivalent to 500 mg/kg was safe and well-tolerated. No harmful effects on growth or hepatic biosynthetic function were observed and stable body weight and albumin suggest otherwise.

*** Recommendations**

More studies are needed to further define the role of glutathione at the metabolic level and in metabolic tissues. Future studies should identify the molecular

mechanisms using investigations like Western blot and RT-PCR to assess the expression of key targets like IRS-1 and the Ob-Rb receptor. Through this strategy, we would determine the intracellular signalling pathways that responsible for the enhanced insulin and leptin sensitivity.

Exploring various dosing options to identify the lowest effective dose, as well as an optimal range that provides adequate antioxidant protection without excessive dosing. Optimizing the dosage would make the supplement more meaningful.

Simultaneously, a histological examination of liver tissue should be incorporated to directly correlate biochemical improvement with hepatocyte integrity. The tissue-level changes can be determined through the use of techniques such as hematoxylin and eosin staining.

For future research, the effects of glutathione in combination with other antioxidants (for example, vitamin E, and alpha-lipoic acid) that could have additive/synergistic benefits on metabolism and hormone should be examined. This strategy may improve treatment efficacy and expand the spectrum of antioxidant-based therapies.

This indicates that translational research is warranted due to the positive result in rats. The conduct of clinical trials in patients with metabolic dysregulation and/or early hepatic stress may enable the evaluation of the therapeutic potential of oral glutathione and facilitate its further clinical development.

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