

EFFECT OF INSULIN TREATMENT ON SOME METABOLIC  
CHANGES ON EXPERIMENTALLY INDUCED TUMOR IN  
FEMALE MICE.

By

**S.A. Hussein and M.E. Azab**

Department of Physiology, Biochemistry and Pharmacology,  
Faculty of Veterinary Medicine, Moshtohor (Benha branch),  
Zagazig University, Egypt.

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ABSTRACT

*Insulin administration in TB-mice caused a very highly significant decrease in the values of plasma glucose, triacylglycerols, free fatty acids, total ketone bodies and urea concentrations. On the other hand, plasma albumin, total proteins and liver glycogen concentrations were markedly increased. Meanwhile, insulin administration in NTB-mice caused a significant decrease in the values of plasma glucose and free fatty acids concentrations. However, the mean values of plasma triacylglycerols and total ketone bodies levels showed a highly significant decrease after insulin administrations. On contrary, the value of plasma lactate, total proteins, albumin and liver glycogen concentrations were significantly increased. The results of this study indicated that, insulin treatment can reverse the experimental cancer cachexia, and it's anabolic therapy, has potential benefits in cancer treatment by shifting glucose metabolism toward the host and away from the tumor.*

INTRODUCTION

Neoplastic disease is associated with a number of metabolic changes including increased gluconeogenesis, muscle proteolysis, and adipose tissue lipolysis which result in host wasting, (Stein, 1978). Cachexia is a frequent accompaniment of cancer. Cachexia has two components: anorexia and distant catabolic effect of tumor. Anorexia is the failure of tumor-bearing host to maintain adequate protein-caloric intake to sustain weight and energy reserves. Distant catabolic effects of tumors include alterations in carbohydrate, protein and lipid

metabolism (Moley et al., 1985). Disordered carbohydrate metabolism is manifested by lactic acidemia (Waterhouse, 1974), abnormal glucose tolerance (Holroyde and Reichard, 1981), increased gluconeogenesis (Burt, et al., 1981), and Cori cycle activity (Holroyde and Reichard, 1981). Tumors grow and incorporate nitrogen at the expense of skeletal muscle protein (Lundholm, et al., 1980) which is also broken down to provide gluconeogenic precursors (Arbeit, et al., 1982). Hyperlipidemia and depletion of fat stores are seen in the presence of tumors (Devereaux, et al., 1984). These metabolic effects contribute to the catabolic decline of the host.

Insulin has anabolic effects that are opposite to many catabolic effects of tumor. Insulin resistance and glucose intolerance have been reported as one of the many biochemical abnormalities in cachectic hosts (Lundholm et al., 1978). Systemic administration of large doses of insulin has been observed to stimulate feeding in anorectic tumor bearing rats (Morrison, 1975) and to increase host weight without affecting tumor growth (Moley, et al., 1984).

However, we have little information concerning possible biochemical anticachectic effects of insulin treatment in anorectic tumor bearing mice.

Accordingly, the purpose of this experiment to elucidate the biochemical alterations of some plasma constituents, body weight and some organ weight of experimentally induced tumor in female mice. It is also valuable to investigate which of these tumor induced biochemical abnormalities could be reversed toward normal by the daily administration of exogenous insulin in tumor animals.

#### MATERIAL AND METHODS

Forty, C57/BL6j Australian female albino mice, 12-16 weeks old, and obtained from the Research Institute of Ophthalmology, Egypt were used in these experiments. Mice were kept at a constant environmental and nutritional condition throughout the period of the experiment. Water was supplied ad-libitum.

#### **Tumor induction:**

The experimental induction of tumor in female mice was carried out at the National Cancer Institute, Egypt. Each mice was injected subcutaneously in the medial aspect of the right thigh with 0.2 ml of Ehrlich ascites adenocarcinoma which contain ( $2.5 \times 10^6$  tumor cells in single cell suspension). The control mice received an equal volume (0.2 ml) of normal saline only (Shapot and Blinov, 1974). The tumor

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developed and become palpable in all injected animals at 5-7 days following tumor inoculation.

#### **Insulin:**

The insulin used in these experiments was Neutral protamine hagedom (NPH) of pH. 7.3. This is suspension of microcrystalline protamine insulin (Manufacturing Division of Nordisk Insulin Laboratorium, Denmark).

#### **Dose:**

2U/100gm total body weight/day for 7 consecutive days. The injections were made subcutaneously after a 1: 10 dilution of insulin and saline was made.

#### **Experimental Design:**

The female mice were divided into four groups, each one consisting of 10 animals placed in individual cages and classified as follows:

- Group I** : Non tumor-bearing control (NTB-C), saline treated.
- Group II** : Non tumor-bearing (NTB-I), insulin treated.
- Group III** : Tumor-bearing control (TB-C), saline treated.
- Group IV** : Tumor-bearing (TB-I), insulin treated.

Fourteen days post-tumor inoculation, tumor-bearing (TB) and Non-tumor bearing (NTB) mice received a single S.C. injection of either saline or insulin 2 units/100g total body weight/day for 7 consecutive days. Average total body weight per group was calculated one day prior and 7 days following saline or insulin injections.

#### **Sampling:**

Heparinized blood samples were collected from all animal groups after sacrificing of mice following 7 days of saline or insulin administration (one day after the last injection). The samples were collected in the morning following an overnight fast. Plasma were separated and processed directly for glucose and lactate determination, then kept in a deep freeze at -20°C until used for subsequent biochemical analysis.

The tumor was dissected free of the surrounding tissue, and their wet weight recorded. The host weight was calculated as (Carcass weight minus tumor weight) (*Moley, et al., 1985*). Moreover, some internal organs as liver, heart and spleen were removed at necropsy

and also weighed, and the relative weight of each organ was calculated as mg organ weight per 10g carcass weight. Also, 0.5g liver specimens were taken, washed with saline, and processed directly for liver glycogen estimation.

**Analytical procedures:**

Plasma glucose concentration was estimated enzymatically with glucose oxidase as described by (Trinder, 1969) and lactate level was determined according to (Gutman and Wahlefeld, 1974). Plasma insulin level was carried out by a radioimmunoassay technique according to (Mullner, et al., 1991). Liver glycogen concentrations were estimated by the method of (Carroll, et al., 1956). Plasma triacylglycerols was determined by (Foster and Dunn, 1973). Non esterified fatty acids by (Duncombe, 1964), and total ketone bodies according to (Pawan, 1958). Plasma urea, total protein, albumin and globulin concentrations were determined also by the methods described by (Lespinas, et al., 1969), (Weichselbaum, 1946), (Bartholomew and Delaney, 1966) and (Doumas and Biggs, 1972), respectively.

**Statistical Analysis:**

The obtained data were statistically analyzed and the significant difference between groups was evaluated by t-test as explained by (Snedecor and Cochran, 1969).

**RESULTS**

The obtained data Table (1) shows the mean values of some biochemical blood parameters concentrations of experimentally induced tumor in female mice and their control. The value of plasma glucose level showed a significant decrease in TB-control mice (Group III) comparatively to NTB-controls (Group I). On the other hand, plasma lactate concentration showed a highly significant increase in TB-control animals than in NTB-control one. A highly significant decrease of plasma insulin level was recorded in TB-control animals in comparison with NTB-controls. Meanwhile, a highly significant decrease of liver glycogen concentration was observed in TB-control mice comparatively with NTB-control one. The mean value of plasma triacylglycerols, free fatty acid acids and total ketone bodies concentrations showed a highly significant increase in TB-control group when compared with the value of NTB-control animals. A highly significant increase of plasma urea level was recorded in TB-control animals, whereas plasma total protein and albumin concentrations revealed a highly significant decrease in TB-control animals in comparison with the NTB-control one. However,

plasma globulin level showed a non significant increase in TB-control animals.

Table (2) shows the effect of insulin administration on some biochemical blood parameters concentrations of experimentally induced tumor in female mice and their control. The mean value of plasma glucose level was significantly decreased in insulin treated animals in both NTB and TB groups (Group II and Group IV), respectively. Plasma lactate levels were highly significantly increased in insulin treated animals in NTB-group, whereas in TB-animals the plasma lactate level was not significantly decreased after insulin administration. A non significant increase of plasma insulin level was recorded in insulin treated animals in both NTB and TB animals. The value of liver glycogen concentration was highly significantly increased in insulin treated animals in both NTB and TB groups.

The obtained results revealed that, there was a highly significant decrease of plasma triacylglycerols, and free fatty acids concentrations in insulin treated animals in TB-control group. However, in insulin treated animals in NTB-group, Plasma triacylglycerols level showed a highly significant decrease whereas, plasma free fatty acids level revealed a significant decrease only. On the other hand, plasma total ketone bodies concentrations were highly significantly lower in insulin-treated animals in both NTB and TB animals (Group II and IV), respectively. A non significant increase of plasma urea level was recorded in insulin-treated animals in NTB-group, whereas in insulin treated animals in TB-group, plasma urea level was highly significantly decreased. Plasma total protein level was significantly increased in insulin treated animals in NTB-group, whereas, in insulin treated animals in TB-group, plasma total protein level was highly significantly increased. On the other hand, a highly significant increase of plasma albumin concentration was recorded in insulin treated animals in both TB and NTB-groups. Moreover, plasma globulin level was non significantly lower in insulin treated animals in both TB and NTB groups.

Table (3) shows the value of body weights in NTB and TB mice before and after saline or insulin administration. The obtained data revealed that, the total body weight of all animal groups showed a non significant increased after saline or insulin administration.

The obtained data Table (4) showed that, there was a significant increase in the relative weights of the spleen and liver of tumor bearing animals when compared with non tumor bearing ones. Meanwhile, insulin administration induce a significant increase in relative

weights of spleen and heart in NTB-insulin treated animals compared with NTB-control animals, whereas, the liver weight was non significantly increased. However, insulin administration in TB-control animals revealed a significant increase in the relative weight of liver and spleen as well as a non significant increase in heart relative weight in comparison with TB-insulin treated animals.

#### DISCUSSION

The obtained data Table (1) revealed that, tumor bearing mice (TB) demonstrated a significant decrease of plasma glucose concentrations. Similar results were reported by (Goodlad, et al., 1975), in tumor bearing rats and (Hobbs and Miller, 1966) in a number of human cancer cases. Also, Shapot and Blinov (1974) observed that, mice with Ehrlich ascites carcinoma developed severe hypoglycemia that increased with tumor growth. Moreover, the same authors recorded that, tumor bearing rabbits exhibited progressive decrease in the blood glucose level. These results indicate that the fall in plasma glucose observed in tumor bearing mice was not due to an overproduction of insulin by either the tumor or the tissues of the host, but is more likely a reflection of the general changes in energy metabolism associated with tumor growth (Begg, 1968). As confirmed by the finding of (Schultze, et al., 1976) who stated that, one postulated mechanism for this hypoglycemic effect of tumor-bearing is increased glucose utilization by tumor itself. In contrary, Moley, et al., 1985 recorded that, plasma glucose levels were similar in tumor bearing and non tumor bearing control rats.

Regarding plasma lactate concentrations, the obtained data demonstrated it's highly significant increase in tumor bearing mice. This result in agreement with (Sauer and Dauchy, 1983) who observed that, plasma lactate increased in tumor bearing animals which is considered, a particularly common finding in cancer. Similarly, (Chance, et al., 1986), reported that, severely anorectic tumor bearing rats exhibited significantly increased lactate concentration. The increase of plasma lactate concentration in tumor bearing mice may be due to decreased hepatic lactate clearance or an increase in tumor glycolysis, (Rofe, et al., 1989).

The obtained data revealed that, there was a highly significant decrease in plasma insulin level in tumor-bearing mice. Similar result was reported by Goodlad, et al., 1975 who observed that, the post absorptive level of insulin was decreased in the serum of tumor bearing rats. Moreover, Mider, 1951 and Bibby, et al., 1987, recorded that, tumor growth was accompanied by progressive hypoglycemia and

a reduction in plasma insulin level in tumor bearing mice. The observed lowering in plasma insulin concentration may be contributed to the catabolic effects of progressive tumor growth. On the other hand, *Burt, et al., 1981* observed no significant difference in insulin level between tumor and non tumor bearing rats.

The present study revealed that, there was a highly significant decrease in liver glycogen concentration in TB-control mice. In the same aspect, the lower hepatic glycogen concentrations was noticed by *Rofe, et al., 1986* in tumor bearing rats. Similar results was also observed by *Shapot and Blinov, 1974* in tumor-bearing rabbits, who reported a depletion of liver and skeletal muscle glycogen. The authors attributed this depletion in the host glycogen reserve to its increased glycogenolysis to compensate the losses caused by the excess consumption of glucose by the tumor.

It is evident from the present study that, plasma triacylglycerols, non esterified fatty acids and total ketone bodies concentrations showed a highly significant increase in tumor bearing control mice. The obtained data agreed well with the results of *Bibby, et al., 1987* who recorded that, an increase in plasma free fatty acids level with minimal (non-significant) and elevation of ketone bodies was noticed in tumor bearing mice. Also, *Goodlad, et al., 1975* reported that, an elevation in serum non esterified fatty acids was observed in tumor bearing rats. Moreover, *Coyle, et al., 1990*, recorded that, tumor bearing rats have a higher plasma ketone bodies and triacylglycerols concentrations than non tumor bearing one. Furthermore, altered lipid metabolism appears to be common in rodent-tumor systems, with observations of increased blood lipaemia and non esterified fatty acids (*Mider, 1951*), decreased lipoprotein lipase activity in adipose tissue (*Beutler, et al., 1985*) and decreased hepatic fatty acid synthesis (*Lanza-Jacoby et al., 1984*) being reported, these observation suggest that the tumor bearing host may exhibit conditions which favour fatty acid mobilization and catabolism, and in the presence of food deprivation and ketogenesis. On the other hand, lipaemia has frequently been observed in tumor bearing animals (*Mider, 1951*), Perhaps reflecting an increased mobilization of lipids to meet increasing energy demands. Moreover, the increase of plasma free fatty acids level may be due to the presence of circulating lipolytic factors, including macrophage derived products such as cachectin (tumor necrosis factor) (*Rofe, et al., 1987*) or tumor derived factors (*Beck and Tisdale, 1987*). As confirmed by (*Rofe, et al., 1987*) who showed that, cachectin increases the lipolytic action of adrenaline in isolated rat adipocytes. The observed hyper ketonemia might be attributed to the hypoglycemia resulting from the

continued demands for glucose by the glycolytic tumor mass, *Rofe, et al., 1988*). Low plasma insulin concentrations would be expected in a hypoglycemic, hyperketonemic metabolic state, and this was observed. Furthermore, the possible mechanisms for the hyperketonemia that was observed in tumor bearing rats are a decreased clearance of ketone bodies in the peripheral tissues, increased hepatic ketogenesis which being due to either an increase in the ketogenic capacity of the liver or increased lipolysis in adipose tissue (*Rofe, et al., 1986*).

The plasma urea level showed a highly significant increase in TB-control mice, whereas, plasma total protein and albumin concentrations revealed a very highly significant decrease. The observed increase in plasma urea concentration in tumor bearing mice may be attributed to the increase in urea production as a result of catabolic effect of tumor. This was confirmed by (*Chance et al., 1986*) who recorded that, many biochemical alterations observed in plasma of anorectic TB rats are consistent with increased catabolism of muscle, redistribution of nitrogen and undernutrition due to reduced concentration of the branched chain amino acids associated with the urea cycle (ornithine, citrulline and arginine) were reduced in tumor bearing rats. The author attributed this reduction in urea to the tumor affinity for nitrogen as indicated by the tumor increased uptake of glutamine and other amino acids, (*Sauer, et al., 1982*). However, the decrease in plasma albumin level recorded in tumor bearing control mice was closely similar to the results reported by *Mitruka and Rawnsley, 1977*. Also, the anorectic TB rats exhibited decreased plasma tryptophan (*Chance, et al., 1986*). Since the majority of plasma tryptophan is normally bound to albumin (*McMenamy and Onclay, 1958*), this decrease in tryptophan is probably due to a reduction in plasma albumin levels (*Chance et al., 1983*). On the other hand, the decrease in plasma total protein level in tumor bearing mice may be either due to the distant catabolic, effect of tumor on host tissue protein which incorporate nitrogen at the expense of skeletal muscle protein (*Lundholm, et al., 1980*) or to the broken down of tissue proteins to provide gluconeogenic precursors (*Burt, et al., 1981*). Whereas, the plasma globulin level showed a non significant increase in tumor bearing mice. However, (*Mitruka and Rawnsley, 1977*) recorded a significant increase in plasma globulin in tumor bearing rats.

The anabolic effects of exogenous neutral protamine hagedorn insulin on tumor-bearing (TB) and non-tumor bearing (NTB) female mice were investigated in Table (2). Exogenous insulin administration produced a similar hypoglycemia in both TB and NTB mice. Similar results were recorded in rats by (*Moley, et al., 1985*). On the other



hand, the obtained results are in partial agreement with the findings of *Rofe, et al., 1989* who recorded that, insulin administration caused a decrease of blood glucose level in NTB but not in TB rats. However, *Chance, et al., 1986* found that plasma glucose level was increased in insulin treated normal rats and decreased in the TB one. The observed hypoglycemia recorded after insulin administration may be resulted from increased glucose entry into muscle and adipose tissue but not to the tumor. Thereby insulin treatment reserves or limits cachexia (*Chance, et al., 1986*).

Plasma lactate concentrations were highly significantly increased in NTB mice, whereas in tumor bearing animals the lactate concentrations as non significantly decrease after insulin administration. The increased plasma lactate levels in insulin treated NTB animals were nearly similar to the results reported by *Chance, et al., 1986* who observed that, plasma lactate level was non significantly higher in insulin treated normal rats. In the liver, insulin inhibits the rate of gluconeogenesis by reducing the activity of the enzymes that promote it. So, the increase in plasma lactate concentrations in insulin treated normal mice might be attributed to the reduced gluconeogenesis as confirmed by *Chiasson, et al., 1976* who observed that, insulin has been reported to reduce the conversion of alanine to glucose in the perfused liver. However, the observed decrease of plasma lactate concentrations in insulin treated tumor bearing mice was nearly similar to *Chance, et al., 1986* who observed that, plasma lactate levels were significantly reduced in insulin treated tumor bearing rats. Since, lactate is a major product of glycolysis in tumor, the author suggested that, insulin reduces aerobic glycolysis in tumor tissue by depriving the tumor of glucose. Thus in the presence of large doses of insulin the available glucose is driven into muscle and relatively less available for tumor use. Moreover, insulin treatment, in addition to increasing glucose uptake in insulin dependent tissues such as adipose and muscles, may also increase pyruvate dehydrogenase activity in all tissues, resulting in enhanced glucose oxidation and a concomitant reduction in lactate production. Insulin also promote glycogen and lipid synthesis from lactate (*French, et al., 1988*) with a concomitant decrease in glycolysis in non tumorous tissues.

The obtained results revealed that, there was a non significant increase in plasma insulin concentrations in both NTB and TB mice after insulin administration. Similarly *Chance, et al., 1986* observed that, twenty four hours after the last insulin administration, plasma insulin levels were still elevated in insulin treated TB and NTB control rats. Also, *Moley, et al., 1985* reported that, exogenous insulin

administration resulted in elevation of serum insulin levels to similar levels in both TB and NTB-control rats.

Regarding liver glycogen concentrations insulin administration caused a highly significant increase in liver glycogen in both NTB and TB animals. This effect of insulin may result from the increase synthesis and storage of glycogen in liver. *Guyton and Hall, 1996*, reported that, insulin increases the activity of enzymes that promote glycogen synthesis, including glycogen synthase.

The obtained results revealed that, insulin administration caused significant decrease of plasma triacylglycerols and non esterified fatty acids, concentrations in both TB and NTB animals. The decrease in plasma free fatty acids may result from the antilipolytic effect of insulin as confirmed by *Beck and Tisdale, 1987* who showed that, insulin could overcome the lipolytic effect of a tumor derived lipolytic factor. Also, *Rofe, et al., 1987* observed that cachectin (tumor necrosis factor) increases the lipolytic action of adrenaline in isolated rat adipocytes. So, in the same aspect (*Fraker and Norton, 1987*) reported that, insulin treatment reverses some of the toxic effects of cachectin (tumor necrosis factor). On the other hand, *Rofe, et al., 1989* found that, insulin treatment did not decrease free fatty acids in TB rats during the initial stage of infusion, which indicate that either the hormone sensitive lipase in adipose tissue is no longer inhibited by insulin or fatty acid uptake by liver and other tissues has been affected. Moreover, the decreased plasma triacylglycerols concentrations after insulin treatment reflect either decreased hepatic uptake and esterification of fatty acids or increased clearance, of triacylglycerols in peripheral tissues, (*Rofe, et al., 1994*).

Insulin administration, resulted in a very highly significant decrease in plasma total ketone bodies concentration in both TB and NTB control animals. Similarly *Rofe, et al., 1989* observed that, ketone bodies decreased in response to glucose or insulin infusions and this was coupled with decrease in free fatty acids which are consistent with decreased hepatic ketogenesis due to either decreased substrate supply or direct inhibitory effects of insulin or glucose on the hepatic metabolism.

The obtained data revealed that, insulin administration resulted in a significant decrease in plasma urea level in TB-animals, whereas plasma total protein and albumin concentrations were increased in both NTB and TB-animals. The observed reduction in urea level after insulin administration may be attributed to the reduction of catabolic effect of tumor tissues by insulin administration as confirmed by (*Felig,*

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1975) who reported that, insulin promotes protein formation and prevent the degradation of protein by increasing plasma amino acids uptake into muscle as well as reducing their efflux from the muscles. Moreover, insulin has also been shown to increase amino acid uptake into muscle and subsequent incorporation into protein (Wool, et al., 1968) as well as decreasing proteolysis. Thus reduced insulin concentration may be contributed to the decrease in muscle and dry carcass weight in MAC 16 tumor-bearing mice. On the other hand, plasma globulin level was non significantly lower in insulin treated animals in both TB and NTB-control mice.

The obtained results showed that, there was a significant increase in the relative weights of the spleen and liver of the tumor-bearing mice compared with non tumor bearing mice. In the same aspect, (Kisner, et al., 1981) observed a depletion of host muscle and adipose tissue, whereas organs such as liver, kidneys, adrenal glands and spleen tend to be spared and may actually enlarged in cachectic cancer patients. This observed increase in the weight of the liver and spleen may be due to production of growth factors by the tumor cells (Ruckebusch, et al., 1991).

The present study revealed that, insulin administration caused significant increase in the relative weights of the spleen and heart in NTB mice, whereas in the TB-mice the relative weights of the liver and spleen were significantly increased. The observed increase in the weight of the previously mentioned organs after insulin administration may be due to anabolic effect of insulin as reported by Ruckebusch, et al., 1991, who reported that, insulin stimulates anabolic reaction that leads to formation of macromolecules in the tissues of many target organs such as liver skeletal muscle and adipose tissues, in addition to other organs as cardiac muscle, aorta and bone surface.

From the obtained results it could be concluded that, insulin treatment can reverse experimental cancer cachexia, and it is anabolic therapy which reducin tumor glycolysis and metabolism, as well as reducing gluconeogenesis and reducing host catabolism. Thus insulin therapy have potential benefits in cancer treatment by shifting glucose metabolism toward the host and away from the tumor.

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Table (1) Mean values of some Biochemical blood parameters of experimentally induced tumor in female Mice and their control.

Parameters	Animal groups	
	Group I (NTB - C) (Mean ± S.E)	Group III (TB - C) (Mean ± S.E)
Glucose (mg/dl)	105.39±8.29	86.01±1.69*
Lactate (mg/dl)	31.61±1.58	47.50±2.23***
Insulin (µu/ml)	8.95±0.18	7.68±0.20**
Liver glycogen (g/100gm wet. wt)	2.62±0.11	0.920±0.03***
Triacylglycerols (mg/dl)	171.15±7.61	255.57±13.10***
Free fatty acids (mg/dl)	26.81±0.99	34.37±1.08***
Total ketone bodies (mg/dl)	2.41±0.23	4.30±0.17***
Urea (mg/dl)	24.57±0.81	28.46±0.27***
Total proteins (gm/dl)	6.21±0.18	4.91±0.07***
Albumin (gm/dl)	3.69±0.17	2.13±0.01***
Globulin (gm/dl)	2.50±0.33	2.78±0.08

Data are presented as Mean ±S.E. ±S. E = Standard error

\*: (P<0.05)

\*\*\*: (P<0.001)

NTB-C→ Non-tumor bearing control (Saline treated)

TB-C→ Tumor bearing control (Saline treated)



Table (2). Effect of insulin administration on concentrations of some biochemical blood parameters in experimentally induced tumor in female mice and their control.

Parameters	Glucose (mg/dl)	Insulin (mU/ml)	Liver glycogen (g/100gm wet wt)	Thyroxine (μg/dl)	Free fatty acids (mg/dl)	Total protein (mg/dl)	Urea (mg/dl)	Urea protein (mg/dl)	Albumin (mg/dl)	Cholin (mg/dl)
Animal groups	( $\bar{x} \pm S.E.$ )	( $\bar{x} \pm S.E.$ )	( $\bar{x} \pm S.E.$ )	( $\bar{x} \pm S.E.$ )	( $\bar{x} \pm S.E.$ )	( $\bar{x} \pm S.E.$ )	( $\bar{x} \pm S.E.$ )	( $\bar{x} \pm S.E.$ )	( $\bar{x} \pm S.E.$ )	( $\bar{x} \pm S.E.$ )
Group I (NTB-C)	103.164 8.296 <sup>bd</sup>	31.614 1.886 <sup>b</sup>	2.624 0.116 <sup>b</sup>	171.156 7.618 <sup>c</sup>	26.834 0.996 <sup>c</sup>	2.414 0.236 <sup>b</sup>	24.074 0.81 <sup>a</sup>	6.274 0.196 <sup>b</sup>	3.094 0.156 <sup>b</sup>	2.86 0.11
Group II (NTB-D)	81.724 4.796 <sup>a</sup>	41.064 1.816 <sup>aa</sup>	3.034 0.026 <sup>aa</sup>	149.074 3.796 <sup>aa</sup>	33.164 0.96 <sup>a</sup>	3.134 0.126 <sup>aaa</sup>	35.664 0.27	6.974 0.36 <sup>a</sup>	4.764 0.226 <sup>aaa</sup>	2.26 0.04
Group III (TB-C)	86.014 1.696 <sup>aa</sup>	47.504 2.316 <sup>aaa</sup>	0.034 0.034 <sup>aaa</sup>	253.574 11.166 <sup>aaa</sup>	34.174 1.066 <sup>aaa</sup>	4.364 0.176 <sup>aaa</sup>	26.464 0.226 <sup>aaa</sup>	6.694 0.026 <sup>aaa</sup>	3.174 0.026 <sup>aaa</sup>	2.86 0.08
Group IV (TB-D)	73.874 1.836 <sup>aaa</sup>	43.114 3.89	1.624 0.146 <sup>aaa</sup>	141.764 5.826 <sup>aaa</sup>	33.864 0.766 <sup>aaa</sup>	2.354 0.126 <sup>aaa</sup>	31.824 0.126 <sup>aaa</sup>	5.594 0.226 <sup>aaa</sup>	3.264 0.126 <sup>aaa</sup>	1.864 0.02

Units are presented as mean  $\pm$  S.E.  $\bar{x}$  (standard error)

The values have the same subscripts in the same column are significantly different at the probability  $\alpha = 0.05$  (p < 0.05),  $\alpha = 0.01$  (p < 0.01)

NTB-C: Non-tumor bearing control (control treated)

TB-C: Tumor bearing control (control treated)

NTB-D: Non-tumor bearing (tumor treated)

TB-D: Tumor bearing (tumor treated)

Table (3). Mean values of live total body weight of non-tumor bearing and tumor bearing female mice before and after 7 days of saline or insulin administrations.

Animal groups	Group I (NTB-C)	Group II (NTB-I)	Group III (TB-C)	Group IV (TB-I)
Body weight				
B.W before saline or insulin administration (g)	23.50±0.78	24.75±1.15	21.45±1.38	22.10±0.99
B.W after saline or insulin administration (g)	23.79±0.66	26.92±1.45	22.31±1.13	23.58±0.99
Calculated loss weight (g)	22.62±0.63	25.51±1.90	19.69±1.13	20.15±0.80
Final tumor weight (g)			1.28±0.47	2.80±0.67

\* live weight loss 90% weight tumor weight.  
 Group I: Non-tumor bearing (saline treated).  
 Group II: Tumor bearing (saline treated).  
 Group III: Tumor bearing (insulin treated).  
 Group IV: Tumor bearing (insulin treated).

Group II: Non-tumor bearing (insulin treated).  
 Group IV: Tumor bearing (insulin treated).

Table (4). Mean values of the carcass weights and relative weights of spleen, heart and liver in different animal groups.

Animal groups	Carcass weight (g)	Spleen weight mg/10g B.W	Heart weight mg/10g B.W	Liver weight mg/10g B.W
Group I (NTB-C)	22.42±0.63	45.32±3.81±80	46.23±2.17±8	392.09±23.30±8
Group II (NTB-I)	25.51±1.89±8*	60.96±1.30±8**	55.07±2.47±8*	453.01±38.16
Group III (TB-C)	19.69±1.19±8*	90.43±8.16±8***c	56.15±4.84	503.81±40.14±8*b
Group IV (TB-I)	20.15±0.99	184.20±22.77±8**	63.19±3.05	679.71±48.62±8*

The values having the same subscript in the same column are significantly different at the probability \*p<0.05, \*\* p<0.01, \*\*\* p<0.001.  
 Group I: Non-tumor bearing (saline treated).  
 Group II: Tumor bearing (saline treated).  
 Group III: Tumor bearing (insulin treated).  
 Group IV: Tumor bearing (insulin treated).