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Effect of kidney tonifying prescriptions- Liu wei di huang Wan and Ba wei di huang Wan on insulin resistance

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Abstract

Metabolic syndrome (MetS) is a cluster of symptoms seen in type 2 diabetes mellitus, cardiovascular diseases, hypertension and obesity. Insulin resistance is a major component of MetS and is thus the key to cure and prevent any related illnesses. The treatment of type 2 diabetes mellitus in Traditional Chinese Medicine is focused on replenishing Yin (fluid) and evacuating fire (heat) from the body and, depending on the symptoms it can also be focused on replenishing the yang. We study how the Kidney tonifying preparations (Liuwei dihuang wan-LW and Bawei dihuang wan-BW) affect osteocalcin levels to treat insulin resistance. We induced insulin resistance in Rats by a large dose of Dexamethasone 1 g/kg/alternate days (DXM) and simultaneously administered the LW low dose 1 g/kg, LW high dose 4 g/kg, BW low dose 1 g/kg, BW high dose 4 g/kg and Simvastatin 20 mg/kg (positive control). After a 2hr oral glucose tolerance test, blood was drawn for serum analysis of lipids, osteocalcin, adiponectin and leptin. Our results showed that LW and BW might act through increasing insulin sensitizing hormones- osteocalcin and adiponectin, and reducing hormones increased in obesity like leptin. The increase in insulin sensitizing hormones might be able to reduce the measures of insulin sensitivity. LW and BW might be unable to control lipid dysregulation in DXM induced insulin resistance.

Keywords: Osteocalcin, Adiponectin, Dexamethasone, Metabolic syndrome.

Introduction

Type 2 diabetes, which was previously referred to as Non-insulin-dependent Diabetes mellitus or NIDDM typically emerges in middle or later life but due to lifestyle changes it now appears earlier and accounts for about 90% of Diabetics worldwide. The International Diabetes Federation has predicted that the number of individuals with diabetes will increase from 240 million in 2007 to 380 million in 2025.[1] Type 2 Diabetes is a heterogeneous disease with a polygenic basis. This type creeps in slowly as the levels of hyperglycemia are unnoticeable at first and so may go undiagnosed for years. Though β- Cells are still present and producing insulin, the quantity is reduced. It is now accepted that β cell mass in Type 2 Diabetes is about 50% of normal and that this reduction is fundamental to Type 2 Diabetes pathogenesis.[2] Insulin resistance usually develops as a prelude and creates a demand for a compensatory increase in insulin secretion which the body cannot sustain. Insulin resistance is defined as a smaller than normal response to a given amount of insulin.[3] Compelling evidence suggests that the development of skeletal muscle insulin resistance is intimately associated with a decrease in osteocalcin. This, and the fact that obesity protects

mammals from osteoporosis and leptin, an adipose derived hormone that had a direct effect on bone through a central pathway, led the researchers to propose that bone remodeling and energy metabolism could be regulated by the same hormone(s).[4] True to this, osteocalcin was shown to increase production of adiponectin, an adipokine produced by differentiating adipocytes. While administration of adiponectin promoted the proliferation, differentiation, and mineralization of osteoblastic cells.[5] According to the five elements theory, the kidney is in charge of bone, and by nourishing one you improve the function of another, pointing to a close relation between bone and glucose.[4] In addition, serum osteocalcin levels were significantly associated with plasma adiponectin levels and inversely related to leptin levels, the presence of metabolic syndrome, and increased insulin resistance independent of age, gender, and BMI.[6] The presence of insulin resistance is observed 10–20 years before the onset of the disease, and is a consistent finding in patients with Type 2 Diabetes.[7] Insulin resistance is also the best predictor of whether or not an individual will later become diabetic.[8] Epidemiological studies suggest that insulin resistance is not only an independent risk factor that induces type 2 diabetes mellitus, but is also a common cause of hypertension, coronary heart disease, and cerebral vascular disease, and is thus the key to cure and prevent heart and cerebral vascular disease. In Traditional Chinese Medicine (TCM) the treatment of diabetes is focused on replenishing Yin (fluid) and evacuating fire (heat) from the body.[9] Kidney nourishing formulas like Liuwei Dihuang (LW) tablets and Bawei Dihuang (BW) tablets have been used to treat diabetes, hypertension, infertility and male impotence. The main compounds in this formula are also known to dispel blood stasis, increase vigor and improve body metabolism.[10, 11] In research LW's antihyperglycaemic activity has been observed in diabetic mice.[12]

It is of paramount importance to establish an insulin resistance animal model, in order to have a better understanding of the pathological process of insulin resistance and to develop therapeutic drugs. The present study was designed to establish an insulin resistance animal model using Wistar rats with more clinically relevant pathophysiological characteristics of insulin resistance based on glucose utility of the body and alterations of various cellular and molecular events related to insulin resistance.[13] Glucocorticoids where shown to induce Type 2 Diabetes, as a side effect, through impairing insulin secretion, β-cell apoptosis, β-cell proliferation and insulin action in vivo. Administration of Dexamethasone 1 mg/ alternate day (DXM) for 4 days resulted in an insulin-resistant state and enabled us to test this hypothesis.[14]

Research Design and Methods

Rat experiment

All experiments were approved by the Tianjin University of Traditional Chinese Medicine Animal Experiment Ethics Committee. The drugs used were obtained from JiuZhitang Inc. (Hunan). 70 Wistar rats (240-280 g) supplied by Beijing HFK Bioscience were acclimatized in communal cages at 24°C, with a 12-hrs light 12-hrs dark cycle for 1 week and had access to a standard chow diet (Beijing HFK Bioscience) and water ad libitum. Rats were then randomly assigned into 7 groups to receive oral administration of either vehicle (distilled water) or Liuwei Dihuang tablets (Low dose-1 g/kg and high dose 4 g/kg/day) or Bawei Dihuang tablets (Low dose-1 g/kg and high dose 4 g/kg/day) and Simvastatin (Merck Sharp) 20 mg/kg/day by gavage for the next 10 days. Simultaneously the rats were given a subcutaneous injection of NaCl (Control) or Dexamethasone (Zhengzhou Pharmaceuticals) 1 g/kg/alternate day (Drug groups). Body weight was recorded at the start and end of the experiment.[15, 16] An oral glucose tolerance test (OGTT) was performed at the end of the treatment period. Rats were fasted for 12 hrs. After measuring fasting glucose concentration in blood obtained by tail incision, using a One Touch Ultra glucometer (Lifescan), the animals were given a glucose solution at 4 g/kg body weight by oral gavage at time 0. Blood glucose was measured after 30, 60.90 and 120 min. Blood was also collected from the abdominal aorta at time 120 min for serum analysis through ELISA (R&D systems) for the hormones, and for the lipids we used commercial test kits (Biosino biotechnology and Science, Beijing, China) .The adrenal glands and liver were excised and weighed.

Statistical Analysis

Data were compared using paired Student's tests or ANOVA as appropriate and are presented as means ±SE. The P level was set at 0.05.

Results

Effect of LW and BW in Dexamethasone injected rats

Administration of Dexamethasone caused a significant decrease in weight compared to the control group (P<0.01) especially in the model group (Fig 1). The Liuwei and Bawei dose groups lost the least weight compared to the model group (P<0.05) while the simvastatin and model groups lost the same amount of weight.

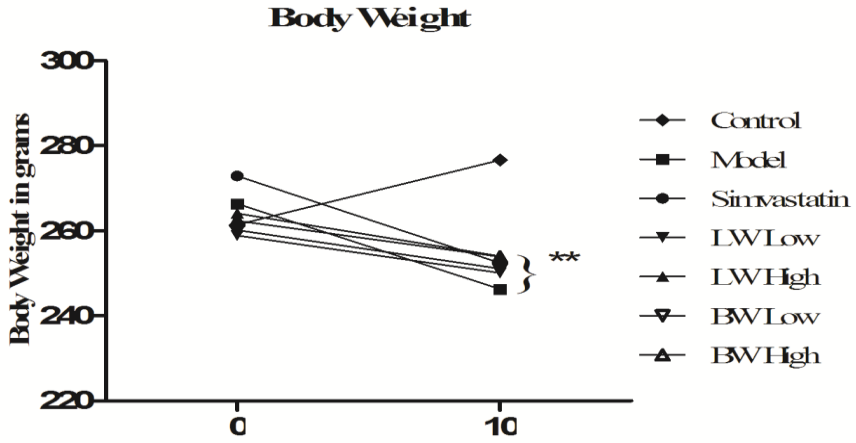

Figure 1: Change in weight after 10 days administration of oral prescriptions with DXM. Black diamond =control, black square=model, black circle=Simvastatin, black inverted triangle= LW Low, black triangle=LW High, white inverted triangle=BW Low, white triangle=BW High. *P<0.05 **P<0.01 compared to control

There was a significant reduction in adrenal weights of the DXM groups compared to the control (P<0.01) with the exclusion of the Bawei High group (Fig 2). The adrenal weights of the drug groups were significantly (P<0.05) higher than the model group with Bawei High group having the greatest significance (P<0.01). The adrenal weights in the Bawei group approached those of the control and were significantly (P<0.01) higher than those of the Liuwei group.

There was an increase in liver weights in the DXM groups (P<0.01) and particularly (P<0.05) for the Liuwei Low group compared to the control, while the Bawei groups were similar to the control and significantly lower than the model group (P<0.01 and P<0.05) for the high and low groups respectively (Fig 2). The liver weights were significantly different (P<0.05) between the Liuwei and Bawei groups.

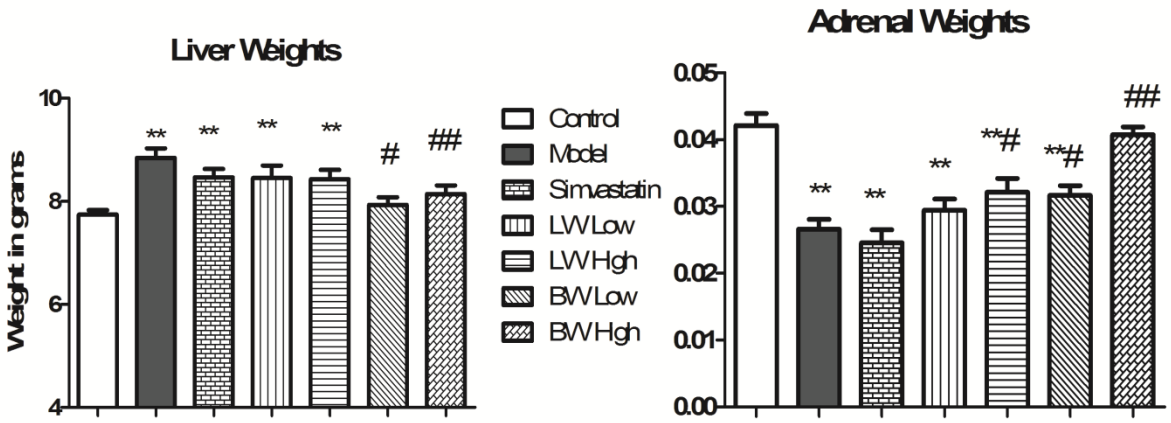

Figure 2: Liver and adrenal gland weights after 10 days administration of oral prescriptions with DXM. *P<0.05, **P<0.01 compared to control while #P<0.05, ##P<0.01compared to model

A glucose curve was generated after a 2 hrs OGTT (Fig 3) in which the peak glucose concentration was at 1 hour and the curve dipped at 2 hrs. The control curve showed a normal dip at 120 min but the DXM groups had a flattened appearance from 60 min onward. The Simvastatin group was significantly (P<0.05) higher than the control,

while the other groups 120 min values were higher than the control but not statistically different.

The area under the glucose curve (Fig 4) increased in most groups but not in a significant way except for the model group in which there was a significant increase in area under the curve (P<0.05). In the DXM group the Liuwei low and Bawei high groups had the lowest area and were significantly lower (P<0.05) compared to the model group.

Osteocalcin concentrations decreased significantly (P<0.01) for all groups after DXM injection except Liuwei low (P<0.05) and Bawei high where an increase was seen with values comparable to those of control group and significantly higher (P<0.01) than the model group (Table 1).

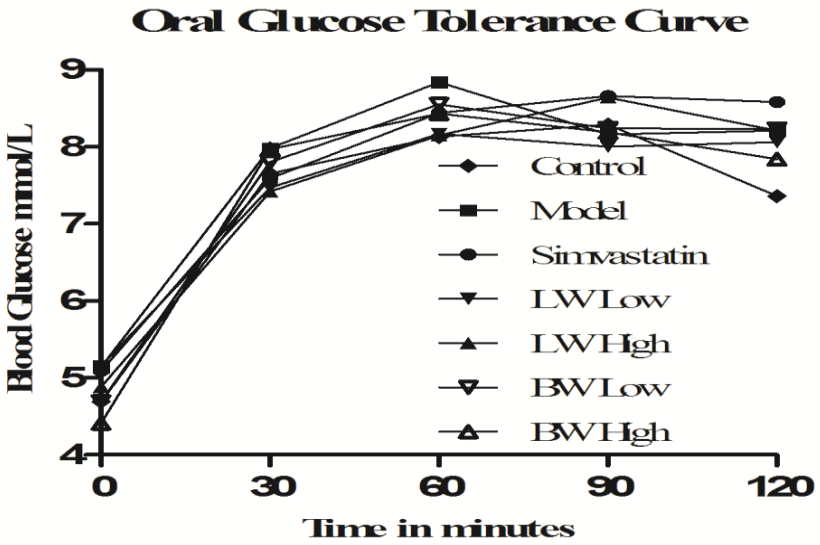

Figure 3: Glucose concentration vs time (min) after 10 days administration of oral prescriptions with DXM. Black diamond=control, black square=model, black circle=Simvastatin, black inverted triangle= LW Low, black triangle=LW High, white inverted triangle=BW Low, white triangle=BW High.

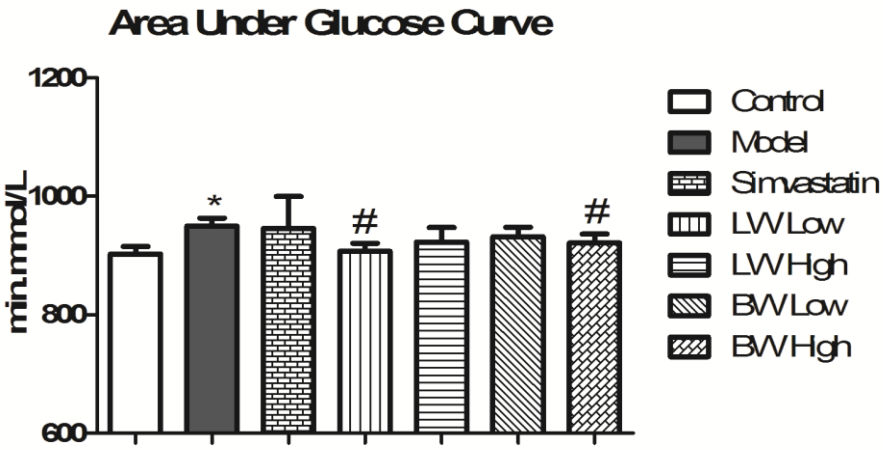

Figure 4: Area under Glucose curve after 10 days administration of oral prescriptions with DXM calculated using the trapezoidal rule. *P<0.05, **P<0.01 compared to control while #P<0.05, ##P<0.01compared to model

Table 1: Hormone and lipid concentrations after 10 days administration of oral prescriptions with DXM. Blood was obtained from the abdominal aorta and serum used to obtain this values using ELISA.

n=10	Control	Model	Simvastatin	Liuwei dh Low	Liuwei dh High	Bawei dh Low	Bawei dh High
Osteocalcin ng/ml	1.17±0.14	1.03±0.09**	1.02±0.13**	1.07±0.10*	1.02±0.12**	0.99±0.14**	1.22±0.20##
Adiponectin ug/ml	1.14±0.12	0.77±0.19**	0.98±0.08***##	0.93±0.05**#	1.01±0.10*##	1.09±0.05##	0.99±0.22#
Leptin ng/ml	1.56±0.15	1.69±0.11*	1.45±0.11##	1.40±0.16*##	1.18±0.11*##	1.26±0.09*##	1.14±0.09**##
Triglyce mmol/L	0.80±0.3	1.72±0.4**	1.98±0.7**	1.81±0.5**	1.86±0.6**	1.80±0.3**	2.10±0.2**##
T Cho mmol/L	1.39±0.3	1.68±0.2*	1.76±0.1**	1.66±0.2*	1.69±0.2**	1.67±0.1**	1.85±0.2**

There was a concomitant decrease in adiponectin concentration which was significant (P<0.01) except for the Liuwei High (P<0.05) and Bawei Low groups. Though the values were lower for the drug groups, they were significantly higher than the model group with P<0.01 for Simvastatin, Liuwei High and Bawei Low groups and a significance of P<0.05 for Liuwei Low and Bawei High groups.

Leptin levels increased significantly in the model group (P<0.05) and decreased in the drug groups except for simvastatin which was nearly similar to the control (Table 1). Compared to the model group all drug groups showed a significant decrease in leptin levels (P<0.01) more so in the high dose groups. This decrease is also seen versus the control group and Liuwei low (P<0.05) and the other drug groups (P<0.01).

Triglyceride values were all elevated after DXM treatment and significantly (P<0.01) more than the control. The Bawei High group triglyceride values were also significantly (P<0.01) higher than the model group.

Cholesterol was elevated in the model and Liuwei low groups (P<0.05) and significantly so in the other drug groups (P<0.01).

Discussion

The pathogenesis of Type 2 diabetes is complex and in most instances involves a defect in both b-cell function and insulin sensitivity. Western therapy usually involves the use of a purified compound acting on one or a few organs or substrates, making it prone to producing side effects.

Administration of high dose DXM causes profound weight loss due to enhanced protein degradation.[17] GCs reduce skeletal muscle mass both by decreasing the rate of protein synthesis and by increasing the rate of protein breakdown as demonstrated by the observed weight loss.[18] Pathogenesis of glucocorticoid induced side effects occurs at the gene level.[19] Recent data suggest that certain side effects are predominantly mediated via transactivation (e.g., diabetes, glaucoma), whereas others are predominantly mediated via transrepression (e.g., suppression of the hypothalamic-pituitary-adrenal axis) or both transactivation and transrepression seem to be involved in GC mediated osteoporosis.[20] Competition of GR and TATA-binding transcription factors occurs at overlapping sites, disrupting the preinitiation complex, and thereby causing the conditional repression of the osteocalcin gene by glucocorticoids[21] as evidenced by the rapid repression, up to 40% of basal levels, of serum osteocalcin concentrations noted after short-term treatment

with glucocorticoids[22, 23] and in syndromes of glucocorticoid excess. Our DXM rats showed a significant decrease in osteocalcin levels after only 10 days of DXM exposure. A decrease in osteocalcin went hand in hand with an increase in AUC, in agreement with Lee et al work on osteocalcin and metabolism.[24] Among the DXM side effects are metabolic derangements, including the development of central adiposity, hepatic steatosis, dyslipidaemia characterised by increased plasma levels of triglyceride rich lipoproteins and nonesterified fatty acids (NEFA)[25], increased breakdown of skeletal muscle mass, insulin resistance, glucose intolerance and overt diabetes in susceptible individuals.[26] This might explain the abnormal findings of the lipid profile we obtained from the DXM rats. The TG's and cholesterol were high, due to the inhibition of GC's on lipoprotein lipase and activation of hormone sensitive lipase, in all of the DXM groups compared to the control.[27] This is better explained by Cole et al who showed that less FA was taken up by livers from DXM treated rats and more triglyceride was synthesized and secreted leading to most of the metabolic derangements observed.[18] Conversion of FA to ketone bodies is also reduced, ensuring high amounts of lipids in the serum. We can hypothesize that chronic elevations of the lipids seen would lead to reduced insulin sensitivity seen after GC administration. BW is known to decrease serum lipid levels, and we hypothesize that this would be effected through an upregulation of genes that favor fatty acid metabolism since we may not have had sufficient time to counteract this effect by the GC. Administration of DXM (1 mg/alternate day) for 4 days resulted in an insulin-resistant state[14], but only a mild one in our case. The AUC and OGTT curve showed impaired glucose tolerance that was not severe. We worked with young rats, 10-12 weeks old and this may have contributed. This is possible since Barbera et al proved it harder to induce insulin resistance in young rats[28], although the site of administration matters since subcutaneous injection of DXM was better at causing muscle wasting, while intraperitoneal injection was superior at inducing insulin resistance.[17] Age dynamics may also play a role since cortisol production is enhanced in the MetS in elderly subjects[29] making them more sensitive to their effects. GC treatment was shown to induce an accumulation of intrahepatic lipids[18] as indicated by the increase in liver TG stores which was inhibited in LW and more so in BW groups. Since the body weights of the DXM rats reduced and the liver weight increased significantly the liver weight: body weight ratio compared to controls is also higher. Also in the LW and BW groups the body weights and adrenal gland weights were better maintained. LW and BW might work to improve insulin resistance by cancelling out the effect of GCs on some tissues.

GCs influence the production of adipocytokines and (adiponectin, leptin and resistin) by affecting ppargamma.[20] The drug groups inhibit the effect of GC in reducing adiponectin which meditate insulin sensitivity and increasing leptin which meditates increased insulin resistance. This is evident in metabolic diseases where the satiety hormone leptin affects not only an adipokine but also Osteocalcin, a bone specific hormone, and correcting one balances the other.[9] Yamauchi et al obtained values with the lipoatrophic mice of increased free-fatty acid (FFA) in serum, increased triglyceride levels, increased tissue triglyceride content in skeletal muscle and liver.[30] This is similar to our DXM rats which are thinner than the control group but are more insulin resistant and have increased serum lipid concentrations. Both in vitro and in vivo in rats, GC treatment was shown to induce the accumulation of intrahepatic lipids fasting plasma non-esterified free fatty acids (NEFA) concentration. Since our rats are thinner but have a higher NEFA concentration, we assumed that the insulin resistance seen is largely unrelated to body fat mass and other factors must be in play.[18]

Despite the deleterious effects of Dexamethasone on osteocalcin and metabolism, the Traditional Chinese Prescriptions seem to work by maintaining the weights and functionality of the organs involved. With long term administration and more research in this area, we might be able to deduce how exactly the prescriptions work to negate the harmful effects of Dexamethasone.

Author Contributions

Y.J.Z. designed and coordinated the study. J.W.K and P.Z. designed collected and analyzed the data. All authors were responsible for interpretation of the data and J.W.K wrote the paper. Y.J.Z reviewed the paper. All authors read and approved the final paper.

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