Insulin Resistance and Hypertension: The Hemodynamic and Metabolic Effects

of Deuterium oxide, Enalapril, and Metformin

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ABSTRACT

Elevated blood pressure has been recognized as a marker for disease since the early 1800s. It is commonly divided into two categories: primary hypertension and secondary hypertension. Primary hypertension is defined as hypertension where inheritable and/or environmental factors are unknown whereas, secondary hypertension is defined as hypertension caused by a known congenital or acquired disease. Primary hypertension is discussed in this paper.

In an attempt to better understand the pathophysiology of hypertension, a common syndrome in patients was described called Syndrome X. Patients with this syndrome have resistance to insulin-stimulated glucose uptake, glucose intolerance, hyperinsulinemia, increased very-low-density lipoprotein triglyceride, decreased high-density lipoprotein cholesterol, and hypertension. In an attempt to better understand the relationship between elevated insulin concentrations (hyperinsulinemia) and elevated blood pressure, experiments were designed using spontaneously hypertensive rats (SHR) as a genetic model of hypertension. Agents which lower blood pressure (deuterium oxide and enalapril) and an agent which lowers plasma glucose concentrations were used to try to elucidate the relationship between insulin resistance and hypertension. If insulin resistance and hypertension are causally related one would expect that by pharmacologically altering one of the abnormalities a similar direction and magnitude of effect would occur in the other.

Two experiments were performed. The first experiment examined the effects of 10% D_2O and 50 mg/L enalapril on hemodynamic and metabolic factors in the SHR.

The second experiment examined a dose range (10, 30, 100, and 300 mg/kg/day) of metformin in SHR and its effects on hemodynamic and metabolic factors. In both experiments body weight, systolic blood pressure, insulin, glucose and triglyceride concentrations in plasma, water intake, and urine volume were recorded weekly. At the end of each experiment direct blood pressures were recorded from the iliac artery.

In the D_2O and enalapril experiment, enalapril significantly lowered the systolic pressure compared to the control and 10% D_2O groups. There was no significant difference in the insulin (mU/L) or glucose (mmol/L) concentrations between the three groups and the insulin:glucose ratio (mU/mmoL) was not significantly different between the groups. These results suggest that there is no effect on insulin or glucose concentrations when the blood pressure is lowered in the SHR.

In the metformin experiment, metformin did not significantly lower the systolic blood pressure during the treatment period. There was also no significant difference in fasting plasma insulin and glucose concentrations. The insulin:glucose ratio also showed no significant difference between the groups.

Conclusions:

1. Ten % D_2O decreases fasting plasma glucose concentrations, thus possible causing a decrease in insulin resistance.

2. Despite this, chronic 10% D_2O has no effect on blood pressure or fasting plasma insulin concentration in the SHR.

3. This suggests that insulin resistance does not cause increased blood pressure.

4. Enalapril decreases blood pressure but has no effects on glucose and insulin concentrations in the SHR, confirming that high blood pressure does not cause insulin resistance.

5. Enalapril causes a large increase in urine volume and water in the SHR.

6. Chronic metformin (10 to 300 mg/kg/day) has no effect on insulin and glucose concentrations or blood pressure in SHR.

7. The SHR may not be an appropriate model for studying the link between hypertension and insulin resistance.

TABLE OF CONTENTS

CHAPTER 1

1. INTRODUCTION

CHAPTER - 2

2. METHODS

CHAPTER - 3

3. RESULTS

CHAPTER - 4

4. DISCUSSION

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGMENTS

1. INTRODUCTION

1.1. Hypertension - A general background

Elevated blood pressure was recognized as a disease entity in 1827 (1). In 1895, Allbutt called a rise in blood pressure without proteinuria "senile plethora", which was later revised to "hyperpiesis" (2). The term "hyperpiesis" was modified to "essentille hypertonie" by Frank, in 1911, and was translated to essential hypertension (3). Today, primary hypertension is most commonly used to describe elevated blood pressure.

In 1955, Pickering characterized primary hypertension as high blood pressure with hypertensive cardiovascular hypertrophy and proposed that it was dependent on inheritance and environment (4). Thus, primary hypertension was thought to be initiated by a polygenic and multifactorial cause. Secondary hypertension was defined as hypertension caused by a known congenital or acquired disease such as renovascular hypertension or primary hyperaldosteronism. This is in comparison to primary hypertension where the inheritable and/or environmental factors are unknown (5).

More recently, primary hypertension has been postulated to be caused by genetic factors (6). This differs from secondary hypertension caused by environmental factors or disease (6-8). There is probably an interaction between environmental and genetic factors such as salt, alcohol, obesity, low exercise, etc. Genetic hypertension is thought to be caused by abnormalities in arterial smooth muscle causing increased peripherial vascular resistance; blood pressure increases steeply at 30 to 50 years of age, without any known environmental factors.

In humans, hypertension can be further divided into four groups on the basis of the blood pressure measurements. Borderline hypertension is defined

by systolic pressure between 140-159 mmHg and diastolic pressures of 90-94 mmHg. Mild and moderate hypertension are defined by systolic pressures of 160-219 mmHg and diastolic pressures of 95-114 mmHg. Lastly, systolic pressures greater than 220 mmHg and diastolic pressures greater than 115 mmHg are indicative of severe hypertension (9).

Hypertension is a particular problem because it is usually asymptomatic and it is the most common cardiovascular disorder in North America, affecting more than 1 in 10 persons (10). It is important to control elevated blood pressure because it can lead to a greater risk of stroke, heart failure, renal disease, peripheral vascular disease, and coronary artery disease (11).

There are a number of methods that are used for lowering blood pressure. These include non-pharmacologic therapies such as restricted sodium intake, weight loss, reduced alcohol intake, and exercise as well as antihypertensive drugs (9).

The pathophysiology of hypertension is complex and not well understood. Recently, Reaven in an attempt to better understand the pathophysiology of hypertension, described a common syndrome in patients called Syndrome X. Patients with this syndrome have resistance to insulin-stimulated glucose uptake, glucose intolerance, hyperinsulinemia, increased very-low-density lipoprotein triglyceride, decreased high-density lipoprotein cholesterol, and hypertension (12). The mechanistic reason for this association between hypertension and hyperinsulinemia has not been elucidated. A review of the relationship between insulin resistance and hypertension follows.

1.2. Hyperinsulinemia and Hypertension

In healthy individuals actions of insulin are numerous but in general insulin is secreted from the pancreas after meals and promotes the storage of carbohydrates, protein, and fat (13). Specific actions of insulin include

increased glucose entry in adipose tissue and muscle as well as an increase in triglyceride deposition in adipose tissue. Insulin is synthesized in the β-cells of the Islet of Langerhans in the pancreas and once it is secreted it has a half-life of approximately 5 minutes. The degradation of insulin mostly occurs in the liver and kidneys but almost all tissues have the ability to metabolize insulin (13). Thus, in normal individuals insulin secretion is triggered by a rise in blood glucose associated with food intake. The release of insulin then promotes glucose uptake into specific tissues where the glucose is metabolized.

Insulin resistance has been described in a variety of ways, but in general it is a reduction in the response to insulin (14). Glucose uptake requires a specific concentration of insulin to promote the transfer of glucose into a cell. Binding of insulin to specific surface receptors triggers unknown intracellular messages which in turn activate glucose transporters that transport glucose across the cell membrane (15). The molecular biology of insulin resistance has recently been described and it has been shown that the impairment of insulin action can be attributed to decreased insulin receptor number and post binding defects of insulin action (16). A malfunction at any stage of this process could result in resistance to insulin-stimulated glucose uptake.

Insulin resistance is present in all patients with non-insulin dependent diabetes mellitus (NIDDM) (12) and is frequently combined with a defect in insulin secretion (17). Obesity is a common precursor of NIDDM and is commonly associated with insulin resistance (17-19). A relationship also exists between NIDDM and hypertension (Syndrome X) (20-22).

A common pathogenic link between diabetes and hypertension may be insulin resistance (20). It has been demonstrated that hypertensive patients, on average, are more insulin resistant than a control population in the absence of obesity or NIDDM (23-25). Bonora, et al. conducted studies involving 247 non-

obese and 120 obese non-diabetic subjects (24). All subjects underwent a standard oral glucose tolerance test which included the measurement of plasma insulin concentrations. One single blood pressure measurement was obtained 1 to 2 hours following the glucose load. Results showed a significant relationship between either systolic or diastolic blood pressure and both fasting and post-glucose plasma insulin. It was suggested that the post-glucose plasma insulin response was independently associated with blood pressure in the non-obese subjects, while the association between plasma insulin and blood pressure in obesity was mainly mediated by factors such as age and body weight. It was concluded that insulin may play a role in the regulation of blood pressure in the absence of obesity (24).

Pollare, et al. studied the relationship between abnormalities in carbohydrate metabolism and hypertension in 143 newly detected hypertensives, which were divided into obese and non-obese groups, and 51 normotensive controls (25). The euglycemic clamp technique was used (initially described by Defronzo, et al. 26) to calculate steady state plasma insulin and glucose concentrations. The non-obese hypertensive group had significantly increased fasting plasma insulin values compared with the control group. The obese hypertensive group had significantly higher plasma insulin values compared to both control and non-obese groups. These results suggest that the abnormalities of carbohydrate, insulin, and lipid metabolism in primary hypertensive patients may occur independently from obesity (25).

In 1985, Mancini, et al. compared obese normotensive and obese hypertensive patients (26). The patients were subjected to an oral glucose tolerance test (OGTT) by giving 75g of glucose as a 33% solution and taking blood samples for glucose and insulin measurements at 0 up to 240 minutes after the glucose load. The results of this study showed a significant increase in

serum insulin in the hypertensive group. The authors also concluded that impaired glucose tolerance was more common in the obese hypertensive group although this finding was based on one data point at 120 minutes after the oral load. It was concluded that in obese patients, high blood pressure was independently associated with impaired glucose tolerance and higher fasting serum insulin levels. The results from this experiment suggest that hypertension and insulin resistance occur independently of obesity, however because of the small number of patients and the other criticisms mentioned above it is not conclusive.

As previously mentioned, Reaven defined the relationship between insulin resistance and hypertension as Syndrome X. Various studies have been carried out since this publication that confirm these results. Glucose clamp studies have shown the presence of insulin resistance in elderly patients with hypertension as compared to normotensive controls (27). Oral glucose loads have been given to middle-aged hypertensive patients who showed exaggerated glucose and insulin responses (28), which could be indicative of insulin resistance. The bulk of data confirms a relationship between these two abnormalities. However, it is not known whether this is a causal relationship or if these two abnormalities develop individually, possibly from an early genetic defect.

The primary site of insulin resistance in hypertensive patients is the skeletal muscle (29,30). Direct measurements in the forearm of hypertensive patients has displayed an impairment in insulin action at the muscle tissue level (29). Julius, et al. postulated that the pressure-induced restriction of the microcirculation, associated with hypertension, would limit nutritional flow and thereby impair glucose uptake in the skeletal muscle. Thus decreased skeletal muscle blood supply may be a possible link between insulin resistance and hypertension (30).

Hwang, et al. conducted an experiment to try to determine if hypertension could be produced by feeding a fructose-enriched diet to normotensive rats (31). The rats were fed the fructose diet for 2 weeks and blood pressure, steady state plasma insulin and glucose, and biochemical measurements were taken. Systolic blood pressure was significantly higher in the fructose fed group in comparison to controls. Hyperinsulinemia and hypertriglyceridemia were also associated with the increase in blood pressure. However, when clonidine (antihypertensive agent) was added to the drinking water the fructose induced hypertension was inhibited, but the increases in plasma insulin and triglyceride were not effected. This evidence suggests that elevated blood pressure is not the cause of the metabolic changes seen in these fructose fed rats.

Furthermore, in response to an oral glucose tolerance test (OGTT) hypertensive men treated with an antihypertensive agent did not show a significant difference in the steady state plasma insulin (SSPI) levels when compared to normotensive and non-treated hypertensive men. This indicates that lowering blood pressure with an antihypertensive agent does not necessarily effect plasma insulin levels (32). Thus, results from both animal experiments and human patients suggest that the metabolic abnormalities associated with insulin resistance are not altered when elevated blood pressure is lowered by antihypertensive agents.

1.3. Genetic Model of Hypertension - The spontaneously hypertensive rat (SHR)

The spontaneously hypertensive rat (SHR) is the most commonly used experimental model of inherited hypertension since its development 27 years ago by Okamoto and Aoki (33). The development of this strain of rat began from inbred Wistar rats, who were sent from the United States to Kyoto University.

These rats (Wistar-Kyoto, WKY) were then outbred within a closed colony and were subsequently screened for elevated blood pressure (34). A single male rat was found with a systolic blood pressure in the range of 150-175 mmHg. There was a subsequent mating of this male rat with a female rat whose systolic blood pressure ranged between 130-140 mmHg, which was above the average of the colony. There was further inbreeding from three generations of offspring until the rats developed blood pressures of > 150 mmHg (34). Okamoto and Aoki defined these rats as arbitrarily hypertensive (33). Blood pressures rose with each generation of hypertension and the development of high blood pressure began to occur at a younger age (34). In 1969, after 20 generations of inbreeding a line of SHRs had become fixed. However, some of these SHRs were released to other laboratories before the 20th generation. Therefore, "genetic variation exists between colonies of SHRs because they either arose from different inbred SHR strains released prior to the strain reaching genetic homogeneity, or because of the genetic drift that is known to occur within and between colonies arising from the same inbred strain" (34). Differences between the colonies are more likely trait difference, which are not related to the development of hypertension (35). Therefore, even though there may be slight differences between colonies, the SHR is an appropriate model for hypertension and may provide clues as to the basis of primary hypertension in humans as explained below.

The similarities of genetic hypertension between man and rat has been explored by Trippodo and Frohlich (36). Certain important differences are recognized; 1) in the human population of primary hypertension, hypertensive patients tend to be heavier than those without hypertension whereas SHR normally weigh less than their normotensive controls, 2) the SHR may have altered thyroid function which does not occur in most primary hypertensive

patients, and 3) the rat has relative resistance to developing significant atherosclerosis, in contrast to primary hypertensive patients whose elevated blood pressure facilitates the onset of atherogenesis (36). The SHR develops increased arterial pressure as early as 3 weeks of age and it continues to increase until approximately 20-28 weeks of age. The onset and rate of development of arterial pressure in humans is not clearly defined.

Despite these differences, the SHR has a number of similarities to human primary hypertension. Increased total peripheral resistance and normal cardiac output are two hemodynamic factors that are found in established hypertension in both humans and SHRs. Moreover, both SHR and human patients display either normal or slightly reduced blood volume, an elevated heart rate, and the progressive development of hypertrophy in the left ventricle. The persistence of vascular resistance in both cases may lead to impaired myocardial function which can result in congestive heart failure. Thus, the hemodynamic alterations in both forms of hypertension appear to follow a very similar course (36).

The participation of renal factors and their effect on arterial blood pressure continues to be studied. Studies have shown that renal blood flow is usually normal or decreased with a normal or slightly reduced glomerular filtration rate and increased filtration fraction in both humans with uncomplicated hypertension (37) and SHR (38). Renal vascular resistance is also elevated in both forms of hypertension (36).

Other similarities such as increased venoconstriction and increased sympathetic nerve activity have been implicated in both humans with primary hypertension and in the SHR, but the exact mechanism of how these factors effect blood pressure in man and rat is still being investigated (36).

Because the similarities outweigh the differences, the SHR is considered to be an appropriate model for studying the mechanism of hypertension in man.

However, is it also an appropriate model for studying the relationship between primary hypertension and insulin resistance? A number of studies have been published which support the view that SHRs develop an insulin resistant state similar to that seen in human hypertensive patients (39-42). In a preliminary report Mondon and Reaven showed that abnormalities in insulin secretion, action, and catabolism existed in rats with spontaneous hypertension (39). This evidence was supported in a later publication which demonstrated cellular resistance to glucose uptake in adipocytes from SHR (40). In 1989, Mondon, et al. suggested that high plasma insulin concentrations (hyperinsulinemia) associated with insulin resistance may be due to a decreased removal of insulin by skeletal muscle and the kidneys rather than impaired hepatic removal of insulin (41). The presence of peripheral insulin resistance in the SHR was observed, specifically in the skeletal muscle (42, 43). Further study of this relationship suggests that SHR release insulin normally, but they exhibit reduced tissue sensitivity to insulin (46) and that this reduced sensitivity is a primary rather than a secondary event in hypertension (47). Experiments by Swislocki and Tsuzuki contribute to the previous findings that SHR are suitable models for insulin resistance and primary hypertension through the expression of insulin resistance in terms of glucose and fatty acid metabolism in SHR (48). Thus it is suggested that the SHR, as well as being hypertensive, may also have metablolic abnormalities.

However, some evidence exists that contradicts the presence of insulin resistance in SHR (44, 45). Gaboury, et al. demonstrated that the action of insulin on glucose metabolism is not impaired in the SHR at a time when their blood pressure is clearly elevated (44). Buchanan, et al. showed no significant difference between SHR and their control strain WKY in the response of skeletal

muscle to insulin (45). Therefore, it is not clear whether insulin resistance is a consistent finding in the SHR.

Does this potential insulin resistant state in the SHR parallel the one seen in human patients? A review by Gerald Reaven concludes that the abnormalities of glucose, insulin, and lipoprotein metabolism that occur in hypertensive patients also occur in the SHR (49). Therefore, it appears that the SHR is the best animal model for studying the relationship between elevated blood pressure and insulin resistance at this time.

1.3.1. The Control Strain - Wistar-Kyoto Rats (WKY)

The Wistar-Kyoto rat (WKY) has been used as the normotensive control strain for the SHR. The WKY differs from the SHR because the increase in arterial blood pressure occurs at a slower rate in the WKY and reaches its maximum at approximately 6-10 weeks of age (36). The mean arterial pressure of the WKY reaches 115-130 mmHg, while the average mean arterial pressure of the SHR is between 190-200 mmHg depending on the colony. However, there is some speculation as to the validity of this normotensive control because it was not developed simultaneously with the SHR (36, 50, 51). Genetic "fingerprint" patterns were examined from both SHR and WKY (50). Results showed that SHR were genetically quite different from the normotensive WKY; only 50% of the DNA fingerprint bands were common between the two strains. Thus, continued comparison of SHR to WKY may have limited value for investigating the pathogenesis of hypertension. Therefore, in the following experiments WKY rats were not studied.

1.4. Pharmacological agents - Deuterium oxide, Enalapril, Metformin

1.4.1. Deuterium Oxide

Deuterium oxide (D_2O) is a stable nonradioactive isotope of water, which has been studied in mammals since the late 1950's. In 1958, a study was conducted in rats to determine the effect of D_2O on glomerular filtration and renal plasma flow (52). The rats were given 50 mole % D_2O as drinking water for 38 days. Results showed a decrease in both filtration rate and renal plasma flow to about 40% of rats on normal water. It was also noted that when the rats were returned to normal tap water the filtration rate and renal plasma flow returned to its normal state. They concluded that the effect of D_2O may have been due to a disturbance of adrenal function. A couple of years later, the effects of D_2O were studied in heart, and voluntary muscle at concentrations varying between 99.8 to 25 % in drinking water (53). D_2O decreased the force and velocity of contraction in both the heart and voluntary muscle. Further investigations in frog muscle, suggested that the contractile proteins could be affected by deuterium (54).

Muscles of the barnacle were used by Kaminer and Kimura to test their hypothesis that calcium release was prevented by D_2O, in the coupling of excitation and contraction (55). Aequorin, a protein which luminesces in the presence of calcium, was used to determine the amount of calcium present in the muscle tissue after exposure to 99.9 % D_2O. The results showed that in the presence of D_2O no calcium was released and therefore no contractile response observed. It has been suggested that D_2O depresses the mobilization of calcium ions by lowering the rate of release of calcium ions, decreasing amount of calcium release, and reducing diffusion of calcium ions (56, 57).

Recent studies with D_2O in Sprague-Dawley rats have demonstrated that D_2O affects vascular muscle relaxation (58). It was suggested that these results occurred through action on the sarcoplasmic reticulum calcium mobilization or

contractile proteins. Therefore, D_2O may have multiple sites of action on vascular smooth muscle.

Deuterium oxide has been shown to affect both insulin and glucose in experimental rats. Experiments with D_2O in Sprague-Dawley rats have shown that 50% D_2O in the drinking water decreases blood glucose over a period of 35 days. It appears that the D_2O treatment slows down gluconeogensis, thus blood sugar cannot be maintained at a normal range (59). It has also been shown that D_2O inhibits insulin release, probably through its stabilizing action on the microtubular system of the β-cell (60). D_2O may also mimic the action of insulin by increasing glucose metabolism in adipose tissue (61). This specific action of D_2O is of interest because D_2O may also promote glucose uptake in the skeletal muscle by acting like insulin at this site.

Vasdev, et al. hypothesized that D_2O may help prevent the development of hypertension, by preventing the abnormal contractile activity of the vascular smooth muscle associated with this abnormality (62). Twenty-five percent D_2O was given to male Dahl salt-sensitive rats for four weeks. The D_2O treatment caused a significant decrease in the systolic blood pressure compared to the non treated rats. It was suggested that the antihypertensive effect of D_2O was the result of increased blockage of calcium channels by bound deuterium ions. A further study showed that D_2O (25%) prevented hypertension in spontaneously hypertensive rats (SHR) compared to their control strain Wistar-Kyoto (WKY) (63). It was also demonstrated that 25% D_2O normalized elevated calcium uptake in the aorta. It was again postulated that the blood pressure lowering effect of D_2O was the result of bound deuterium ions in the vascular calcium channels.

An investigation of the dose-dependent effect of D_2O in drinking water on systolic blood pressure and aortic calcium uptake was conducted in SHR to

determine the minimum effective dose of D_2O (64). SHR were treated for 7 weeks with 5%, 10%, and 20% D_2O. 10% and 20% D_2O prevented the increase in systolic blood pressure. These two groups also displayed normal values of aortic calcium uptake. It was concluded that 10% D_2O was the minimum dose required to completely prevent the development of hypertension and elevated aortic calcium uptake in SHR. Because of its potential effect on both glucose metabolism and blood pressure we decided to use 10% D_2O as an antihypertensive agent to try to determine the relationship between elevated blood pressure and insulin resistance.

1.4.2. Enalapril

Enalapril is an angiotensin-converting enzyme (ACE) inhibitor and is one of the drugs currently available for the treatment of hypertension (65). It is a "prodrug" and is converted into the active compound enalaprilat by the liver. The production of angiotensin II (Ang II) is prevented by enalapril, thus preventing the pressor action of Ang II on the arteriolar smooth muscle (66). There is a decrease in arteriolar resistance and arteriolar pressure. There is also a decreased production of aldosterone because of the lack of Ang II action to increase aldosterone secretion. The lack of aldosterone prevents sodium retention in the renal tubule which, along with the lack of arteriolar constriction, causes a decrease in blood pressure (66).

Enalaprilat, once converted from enalapril, has a greater affinity for the angiotensin-converting enzyme than its predecessor, captopril (67). It is rapidly absorbed from the gastrointestinal tract and reaches peak serum concentrations in about one hour. Its half-life is approximately 11 hours, which is more than twice as long as the half-life of captopril. Because of the time needed for hydrolysis by the liver to convert it to its active form, the onset of action of

enalapril is slow (two to four hours). The excretion of enalapril and enalaprilat is unchanged in the urine.

The efficacy of ACE-inhibitors in hypertensive patients has been well documented (65-67). The antihypertensive effects of this class of drugs are also seen in the spontaneously hypertensive rat (68-70). ACE-inhibitor treatment in young SHR for 4 weeks was sufficient to prevent the full expression of genetic hypertension (68). As in humans, ACE-inhibitors exert their antihypertensive effect in SHR by blocking the renin-angiotensin system (69). Enalapril, at a dose of approximately 25 mg/kg/day in the drinking water, significantly reduced mean arterial pressure in the SHR compared to the control group receiving normal tap water (70). Therefore, enalapril was used in our experiments to compare it's antihypertensive effects to the antihypertensive effects of deuterium oxide.

1.4.3. Metformin

Metformin is an oral hypoglycemic agent, widely used in Europe and Canada for the treatment of NIDDM. It is composed of two guanidine molecules that are linked together with the elimination of an amino group, thus it is in the drug class of the biguanides with other agents such as buformin and phenformin (79). Metformin is not metabolized. Its absorption is slow (approx. 6 hours) and it is excreted in the urine at a renal clearance rate of about 450 mL/min. Metformin has a rapid elimination in humans, with a half-life between 1.7 and 3 hours and plasma concentration at a steady state ranges from about 1 to 2 μ g/mL (80).

Metformin differs from sulfonylureas in a number of various respects. It does not undergo biotransformation and is not bound to plasma proteins. It is eliminated solely by the kidney and rarely causes hypoglycemia. Generally, it does not cause weight gain. The doses of metformin given range from 500 to 1000 mg, up to three times a day and it is usually given with meals (80).

The mechanism of action of metformin is not completely understood. However, studies show that it does not stimulate the release of insulin (81-85) as do the sulfonylureas. Metformin has been shown to reduce basal hepatic glucose production and improve oral glucose tolerance without increasing glucose uptake in patients with NIDDM (80). It also improves insulin-induced whole-body glucose uptake in these patients. Metformin causes a decrease in fasting blood glucose, insulin and C-peptide concentrations in plasma (86). It also increases insulin action at the cellular level (83) without raising the plasma insulin concentrations (82).

A number of studies have attempted to elucidate the mechanism of action of metformin. One suggestion is that metformin's action is due to a post receptor event that causes glucose lowering and that the effects of metformin on insulin binding are indirect (87). It is also postulated that the basis for the hypoglycemic effect of metformin is at the level of the skeletal muscle, where it increases glucose transport across the cell membrane (88). In muscle cells, metformin has been shown to stimulate specific glucose transporters; GLUT1 and GLUT4 (88-90). Metformin has also been shown to increase insulin stimulated glucose transport by potentiating GLUT1 and GLUT4 transporters in the plasma membrane in rat adipocytes (89). It has been suggested that the increased glucose uptake caused by metformin results from an increase in glucose transporter number without the need to invoke a modification of intrinsic transporter activity (90). These results suggest that metformin stimulates glucose transport in muscle cells independently of insulin. Therefore, insulin and metformin may be exerting their effects through different subcellular pathways (90). Although the effects of metformin on glucose transporters enhances our knowledge of its mechanism of action, it is still not fully understood how metformin lowers plasma glucose.

Due to its ability to lower plasma glucose levels without increasing insulin levels, metformin has been used to study the relationship between insulin resistance and hypertension. The effect of metformin on blood pressure and metabolism was studied by Landin, et al. in nine non-obese men with hypertension to try to determine the role of insulin resistance (91). They showed that metformin treatment (30 mg/kg/day) significantly lowered blood pressure after six weeks. Two months after the removal of the drug the blood pressure increased, suggesting insulin resistance plays a role in the etiology of hypertension. Spontaneously hypertensive rats were also treated with metformin (200-250 mg/kg/day intraperitoneal) and a significant reduction in MAP (control; 142±6, metformin; 125±4) was observed after seven days (92). Therefore, we decided to examine these effects of metformin in the SHR for an extended treatment period (10 weeks). Given the usual dose of metformin in patients is 30 mg/kg/day, four doses at 10, 30, 100, and 300 mg/kg/day, were used to study the effects of chronic metformin treatment in SHR.

1.5. Objectives

To try to elucidate the relationship between insulin resistance and hypertension using the spontaneously hypertensive rat by studying agents which primarily lower blood pressure (deuterium oxide, enalapril) and possibly have an effect on glucose metabolism and an agent which primarily lowers plasma glucose levels (metformin) and possibly has an effect on blood pressure. If insulin resistance and hypertension are causally related one would expect that by pharmacologically altering one of the abnormalities a similar direction and magnitude of effect would occur in the other.

2. METHODS

2.1. Deuterium oxide and Enalapril

Animals:

Twenty-four male spontaneously hypertensive rats (SHR) were obtained from Charles River Canada (200-220g). These rats were maintained on a 12/12h light/dark cycle and food and water were available *ad libitum*. For one week prior to experimental onset the rats were acclimatized to restraining tubes for subsequent blood pressure measurement via tail cuff (approximately 15 min./day).

Experimental Setup:

At eight weeks of age ($\approx$ 200g), the 24 male SHR were randomly divided into three groups: control, 10% D_2O, and 50 mg/L enalapril (n=8). Drug treatment was given through the drinking water a by single-blind experimental protocol. Water bottles were filled and coded by an individual who was not involved in any of the measurements. In addition all plasma analyses were done on coded samples. During the six week treatment period body weight, urine volume, and water intake were recorded weekly. Blood samples were taken via the tail and systolic blood pressure was measured by tail cuff weekly. Systolic blood pressure was taken in the morning (09:00 - 12:00) and all blood samples were also taken in the morning following a 12-14 hour fast. After the six week treatment period direct blood pressure was measured by iliac artery cannulation under pentobarbital anesthesia (0.1mg/100g). An intracardiac blood sample was obtained for plasma biochemistry prior to sacrifice.

Measurements/Analysis:

Weekly blood samples (0.5mL) were collected by loosely wrapping each rat in a towel, to restrict movement, with their tail exposed. Approximately 1mm of the tail tip was cut-off to allow for bleeding. Blood was collected in 1.0mL eppindorf tubes which were coated with heparin. The tail tip was subsequently submerged into a 3% hydrogen peroxide solution for antiseptic purposes. Blood

samples were then centrifuged for 10-15 min. and the plasma removed and transferred to a second tube and stored at -20°C for future use.

Fasting plasma samples were analyzed for insulin (Immuncorp Inc.), glucose (Sigma Co.), and triglycerides (Sigma Co.) with diagnostic kits. The ratio of insulin to glucose (mU:mmol) was used from each rat as an indicator of insulin resistance.

The SHR were housed overnight (12-14h) in metabolic cages for measurement of water intake and urine output. Urine samples were analyzed for D_2O content with a single-beam infrared spectrometer (MIRAN 1FF, The Foxboro Co.) and for sodium and potassium levels with a flame photometer.

Systolic blood pressure was recorded indirectly from the tail artery using a pneumatic pulse transducer (Narco Bio Systems Inc.). Blood pressure was recorded as an average of three measurements.

Cannulae were inserted into the iliac artery while rats were under pentobarbital anesthesia. Systolic and diastolic pressure were recorded after a 10 minute wait to allow for pressure stabilization.

2.2. Metformin

Animals:

Twenty-four male spontaneously hypertensive rats (SHR) were obtained from Charles River Canada (200-220g). These rats were maintained on a 12/12h light/dark cycle and food and water were available *ad libitum*. For one week prior to experimental onset the rats were acclimatized to restraining tubes for subsequent blood pressure measurement via tail cuff (approximately 15 min./day).

Experimental Setup:

Preliminary experiments with 10 mg/kg/day metformin followed the same experimental protocol as below.

At eight weeks of age ($\approx$ 200g), the 24 male SHR were randomly divided into four groups: control, 30 mg/kg/day, 100 mg/kg/day and 300 mg/kg/day metformin (n=6). Drug treatment was given through the drinking water. During the 10 week treatment period body weight, urine volume, and water intake were recorded weekly. Blood samples were taken via the tail and systolic blood pressure was measured by tail cuff weekly. Systolic blood pressure was taken in the morning (09:00 - 12:00) and all blood samples were taken in the morning following a 12-14 hour fast. After the 10 week treatment period direct blood pressure was measured in concious rats by an iliac artery cannula (see below). This technique was used to eliminate the effect of the anesthetic on blood pressure as was observed in the first experiment.

Measurement/Analysis:

Weekly blood samples (0.5mL) were collected by loosely wrapping each rat in a towel, to restrict movement, with their tail exposed. Approximately 1mm of the tail tip was cut-off to allow for bleeding. Blood was collected in 1.0mL eppindorf tubes which were coated with heparin. The tail tip was subsequently submerged into a 3% hydrogen peroxide solution for antiseptic purposes. Blood samples were then centrifuged for 10-15 min. and the plasma removed and transferred to a second tube and stored at -20°C for future use.

Fasting plasma samples were analyzed for insulin as described in 2.1.

The SHR were housed overnight (12-14h) in metabolic cages for the measurement of water intake and urine output.

Systolic blood pressure was recorded indirectly from the tail artery using a pneumatic pulse transducer (Narco Bio Systems Inc.). Blood pressure was recorded as an average of three measurements.

For direct blood pressure measurements, cannulae were inserted into the iliac artery while rats were under halothane anesthesia. Two small incisions

were made, one in the back of the neck between the ears and the other in the thigh region above the iliac artery. A cannula was then run subcutaneously from the neck to the thigh region. The iliac artery was exposed and cannulated. Both incisions were sutured and the rats were placed back in their cages. After a 24 hour recovery period, direct systolic and diastolic pressures were recorded. The protruding cannula at the neck was attached to a Grass transducer and direct blood pressure was recorded after a 10 minutes wait to allow for pressure stabilization. After the direct blood pressure was recorded the rats were reanesthetized and the chest wall retracted to expose the heart for an intracardiac puncture ($\approx$ 2mL of plasma was collected).

2.3. Statistics

A one-way ANOVA and an unpaired two tailed student's t-test were used to compare direct blood pressure between the groups. A repeated measures ANOVA with Duncan's multiple range test was used to compare the weekly differences in body weight, urine volume, water intake, insulin, glucose, triglycerides, and indirect systolic blood pressures. $P < 0.05$ was accepted as a significant difference and all results are recorded as mean $\pm$ S.E.M. All experimental methods were pre approved by the Animal Care Committee of U.B.C.

3. RESULTS

3.1. Deuterium oxide and Enalapril

Body weight was not significantly different between the three groups, although there was a significant increase in weight during the six treatment weeks ($p < 0.05$, figure 1). Systolic blood pressure (mmHg) rose in the control group from 139$\pm$2 to 154$\pm$10 and this increase in blood pressure was not significantly prevented by 10% D_2O (132$\pm$4 to 161$\pm$5) but was prevented by

enalapril (127±6 to 113±6, p < 0.05, figure 2). The direct blood pressure (mmHg) measurements by iliac artery cannulation also confirm that the systolic and diastolic blood pressure of the enalapril group (133±2/92±4) was significantly lower than both the control (156±11/107±11) and 10% D_2O groups (144±8/97±6) (figure 3).

The enalapril group had significantly higher 12 hour water intake (44±3 mL) compared to the control (20±3 mL) and the 10% D_2O (17±4 mL) groups (figure 4). Urine output (mL) was also significantly higher over the six week treatment period in the enalapril (34±1) compared to the control (16±2) and 10% D_2O (13±1) groups (figure 5). There were no significant differences in weekly urine potassium excretion (mmol/12hrs, p < 0.05, figure 6) between the three groups whereas, the urine sodium (mmol/12hrs) was significantly lower in the 10% D_2O group (0.24±0.01) compared to both the control (0.36±0.02) and enalapril (0.42±0.06) groups (p < 0.05, figure 7). The measurement of D_2O in the urine by single-beam infrared spectrometry demonstrated a gradual increase in the D_2O level until it reached a plateau of 8% at 11 weeks of age (figure 8).

The glucose measurements (mmol/L) taken during the 7 week treatment period showed no significant difference between the control (7.8±0.2) group and the treatment groups (figure 9). However, the 10% D_2O group and the enalapril group were significantly different from one another, 7.2±0.2 and 8.2±0.2, respectively. The triglyceride levels (mmol/L) were significantly decreased in the 10% D2O (0.43±0.04) and enalapril groups (0.44±0.03) as opposed to the control group (0.51±0.05) (figure 10). There was no significant difference in insulin levels (mU/L) between the control (62.6±6.4), 10% D2O (56.3±4.9), or enalapril (52.7±3.8) groups (figure 11). The insulin:glucose (mU:mmol) ratio also showed no significant difference between the groups (control, 7.2±0.6; 10% D2O, 7.0±0.3; enalapril, 6.1±0.3, figure 12).

3.2. Metformin Experiment

Preliminary experiments using 10 mg/kg/day metformin showed no significant difference in body weight, systolic blood pressure, water intake, urine volume, fasting plasma insulin, fasting plasma glucose, fasting plasma triglycerides, nor in the insulin glucose ratio between the control and treated groups. These results led to choosing a higher dose range (30, 100 and 300 mg/kg/day metformin) to determine the dose-response relationship of metformin in lowering blood pressure and increasing insulin sensitivity in the SHR

Body weight was not significantly different between the four groups (control, 30, 100, and 300 mg/kg/day metformin, $p < 0.05$) during the 10 week treatment period, although there was a significant increase in weight over the duration of the experiment (figure 13). The systolic blood pressure was not significantly different between the control (156±3) and treatment groups (154±3, 158±4, 160±4; 30 mg/kg/day, 100 mg/kg/day, and 300 mg/kg/day, respectively) throughout metformin treatment (figure 14). The direct blood pressure measurements taken at week 11 showed a significant decrease in systolic and diastolic blood pressure in the 300 mg/kg/day metformin treated group (166± 9/98±11) compared to the control (185±8/117±8) and other two treatment groups (192±7/122±4, 198±5/128±8; 30 and 100 mg/kg/day, respectively) (figure 15, Table 1).

The control group was not significantly different from the treated groups in 24 hour water intake ($p > 0.05$, figure 16). The 24 hour urine volume also displayed no significant difference ($p > 0.05$) between the control and treatment groups (figure 17).

Fasting plasma glucose (mmol/L) was monitored throughout the 10 week metformin treatment and indicated no significant difference between the control (5.7±0.2) and treatment groups (5.6±0.2, 5.7±0.2, 5.7±0.1, 30, 100 and 300

mg/kg/day metformin, respectively, figure 18). The final fasting glucose levels at week 11 showed that the treatment group receiving 300 mg/kg/day of metformin (9.0±1.5) had significantly higher fasting glucose levels than the control group (6.8±0.4) whereas the 30 and 100 mg/kg/day metformin treated groups (7.1±0.8 and 6.6±0.7, respectively) were not significantly different from control (Table 1). Measurement of the fasting plasma triglycerides (mmol/L) showed no significant difference between the four groups (0.56±0.02, 0.59±0.02, 0.51±0.02, 0.5±0.02, control, 30, 100, 300 mg/kg/day metformin, respectively, $p > 0.05$, figure 19). This was also verified by the final plasma analysis for triglycerides which also showed no significant difference between the control and treatment groups (Table 1). Fasting plasma insulin levels (mU/L) between the control (93±6) and treatment groups (133±29, 89±3, 138±27, 30, 100, 300 mg/kg/day metformin respectively, $p > 0.05$) were also not significantly different from one another (figure 20). However, the final plasma samples show that the group treated with 300 mg/kg/day metformin had significantly higher plasma insulin levels than the other three groups (Table 1). The insulin:glucose ratio (mU/mmol) between the four groups was not significantly different during the 10 week metformin treatment (figure 21). However, the final plasma samples show that the group treated with 300 mg/kg/day metformin had a significantly higher insulin:glucose ratio than the control and other two treatment groups (Table 1).

Figure 1. Body weights (g) measured weekly. There was no significant difference between the control, 10% D_2O, and 50mg/L enalapril groups ($p >$ 0.05). Values are means $\pm$ S.E.M., n = 8 for each group.

Figure 1. Weekly Body Weight

Weight (g)

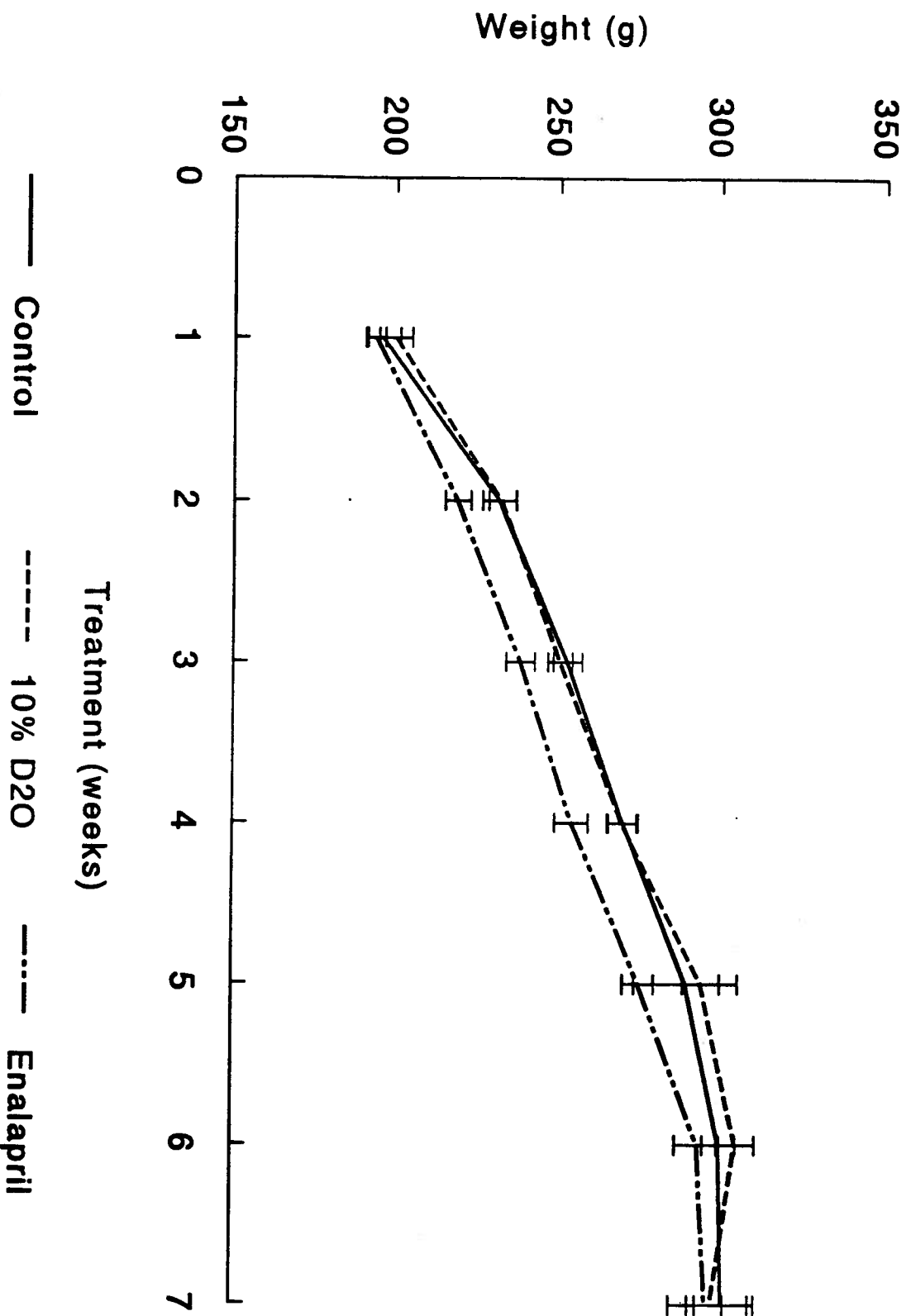Treatment (weeks)

——— Control   —---— 10% D2O   —--— Enalapril

Figure 2. Indirect systolic blood pressures (mmHg) measured weekly via tail cuff. There is no significant difference between control and 10% D_2O, while the enalapril group is significantly lower than both groups. Values are means ± S.E.M., n = 8 for each group. * indicates a significant difference (p < 0.05) from control and 10% D_2O.
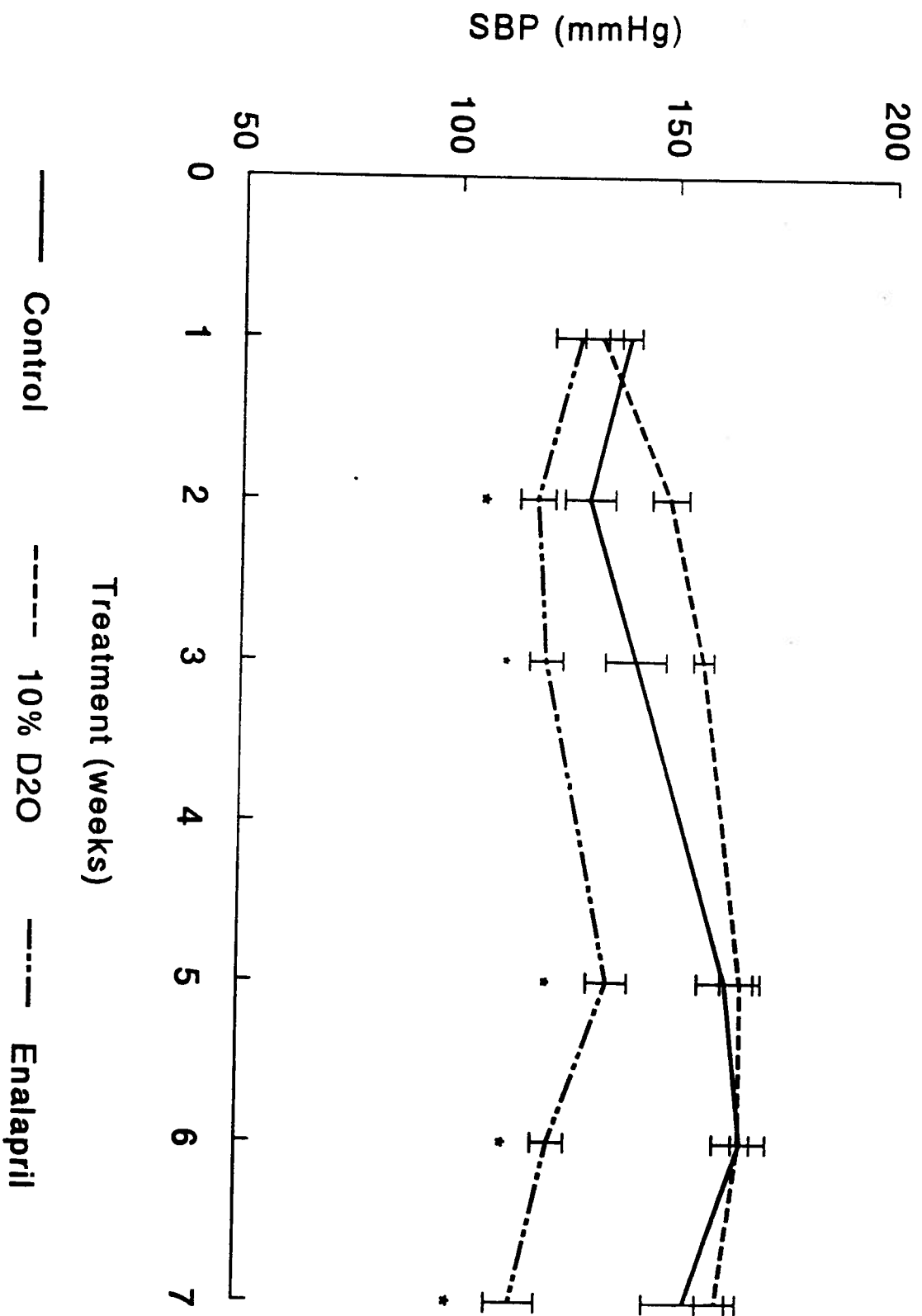
Figure 2. Systolic Blood Pressure

Figure 3. Direct blood pressure (mmHg) recordings after six weeks of treatment. There is no significant difference in systolic pressure (top of bar) between control and 10% D_2O, while enalapril is significantly different from both groups (p < 0.05). There is a significant difference in diastolic pressure (bottom of bar) between control and enalapril. Values are means ± S.E.M., n = 8 for each group. * indicates a significant difference (p < 0.05) from the control and 10% D_2O. ** indicates a significant difference from control.
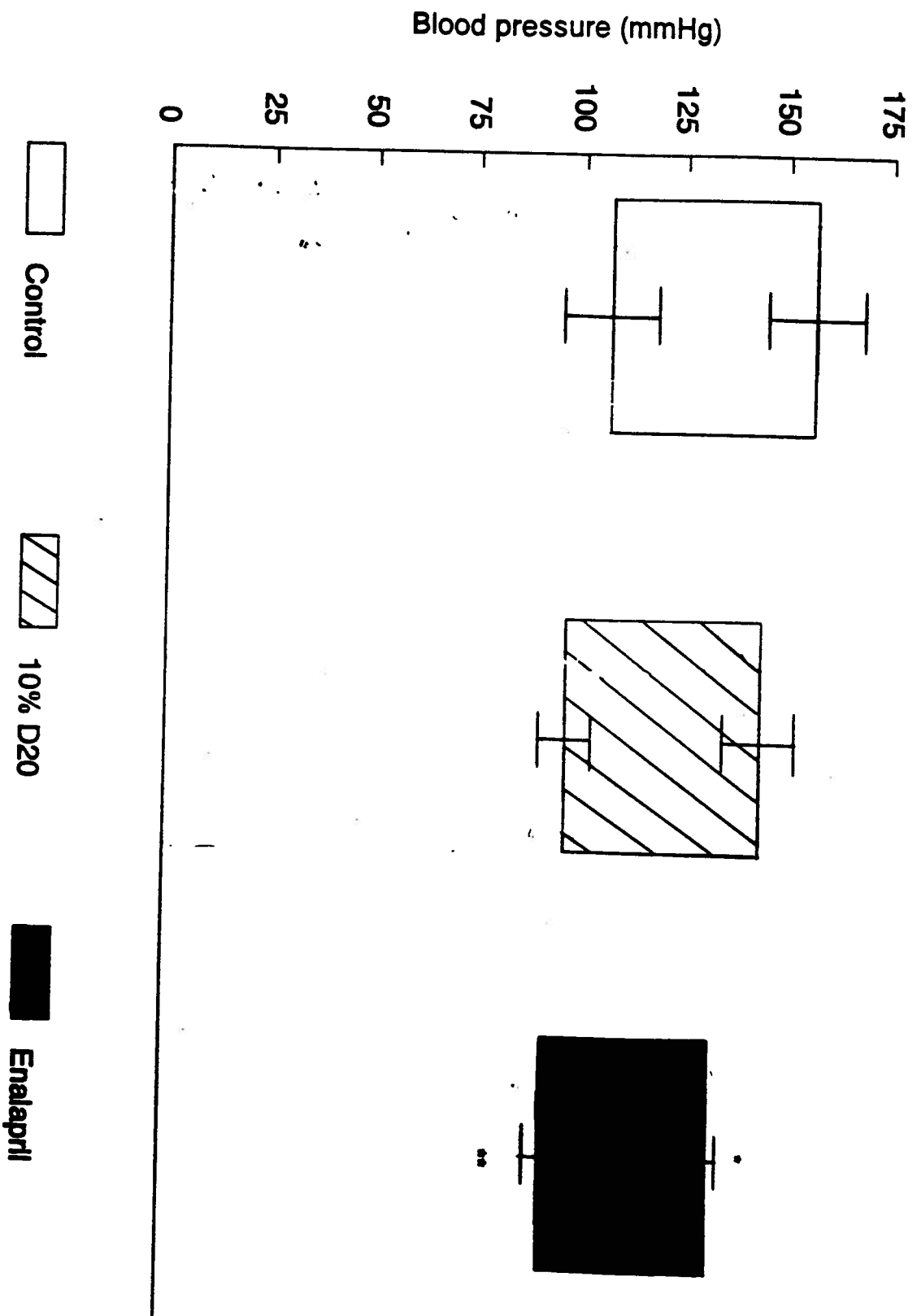
Figure 3. Direct Blood Pressures

Figure 4. Twelve hour water intake (mL). No significant difference was found between the control and 10% D_2O groups, while the enalapril group was significantly different from both. Values are means $\pm$ S.E.M., n = 8 for each group. * indicates a significant difference (p < 0.05) from both control and 10% D_2O.

Figure 4. Twelve Hour Water Intake

Water Vol (mL)

Treatment (weeks)

——— Control
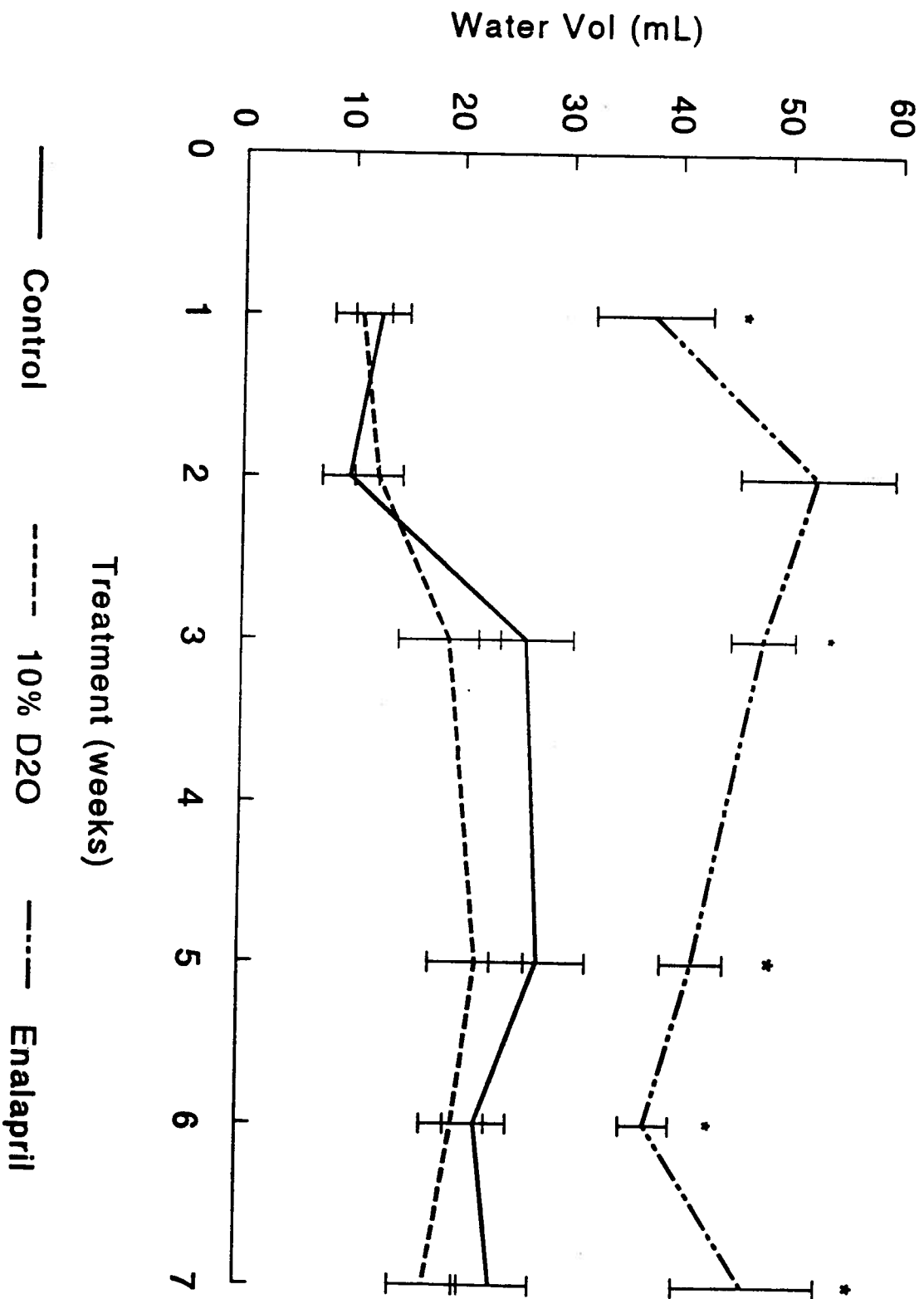
- - - - 10% D2O

—-—- Enalapril

Figure 5. Weekly urine volumes (mL) measured over 12 hours. There was no significant difference between the control and 10% D_2O groups, while the enalapril group was significantly different from both. Values are means ± S.E.M., n = 8 for each group. * indicates a significant difference (p < 0.05) from both control and 10% D_2O.

Figure 5. Twelve Hour Urine Volume

Figure 6. Urine potassium (mmol/12h) measured weekly multiplied by the 12 hour urine volume. There is no significant difference (p > 0.05) between the control, 10% D_2O, and enalapril groups. Values are means ± S.E.M., n = 8 for each group.
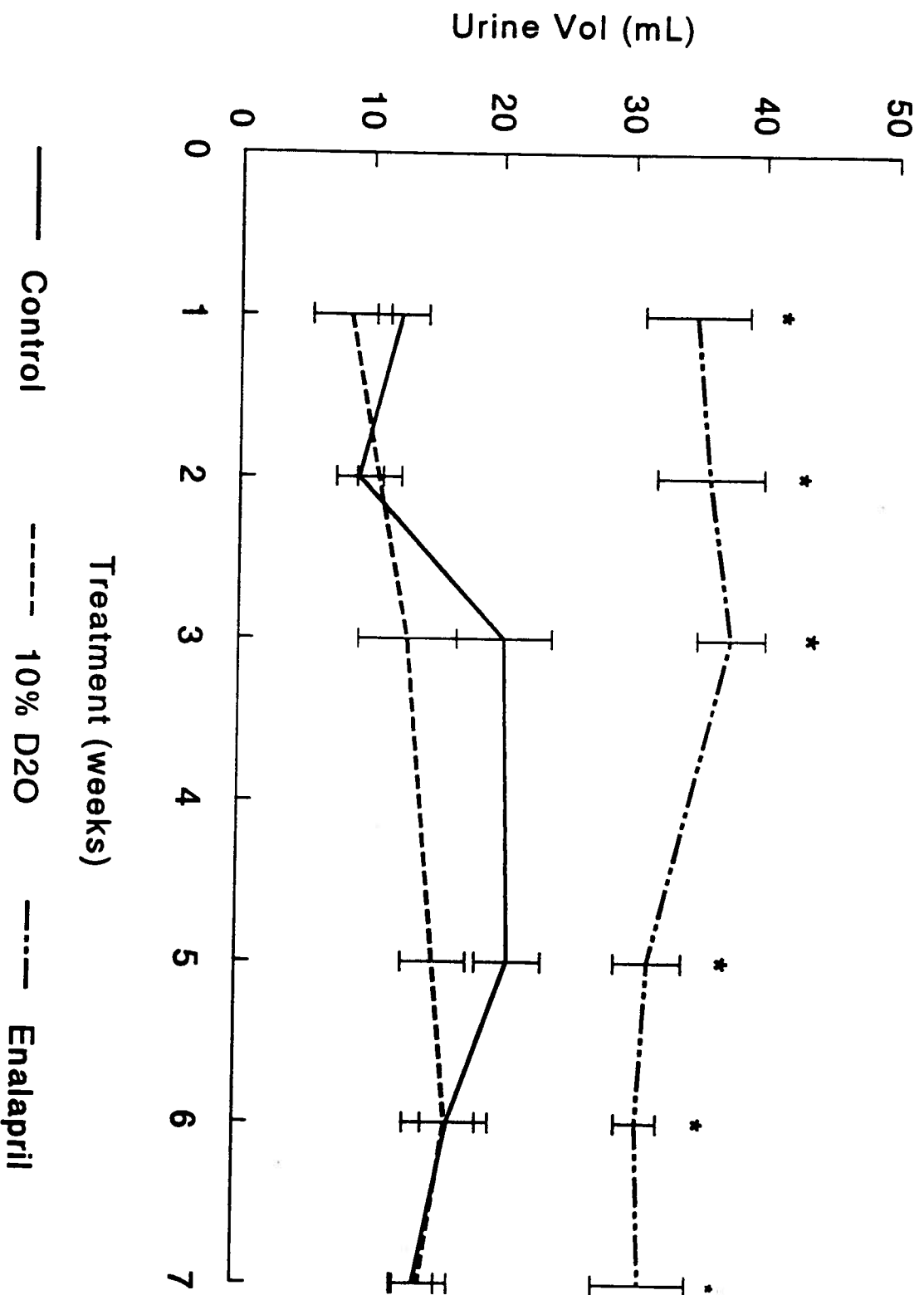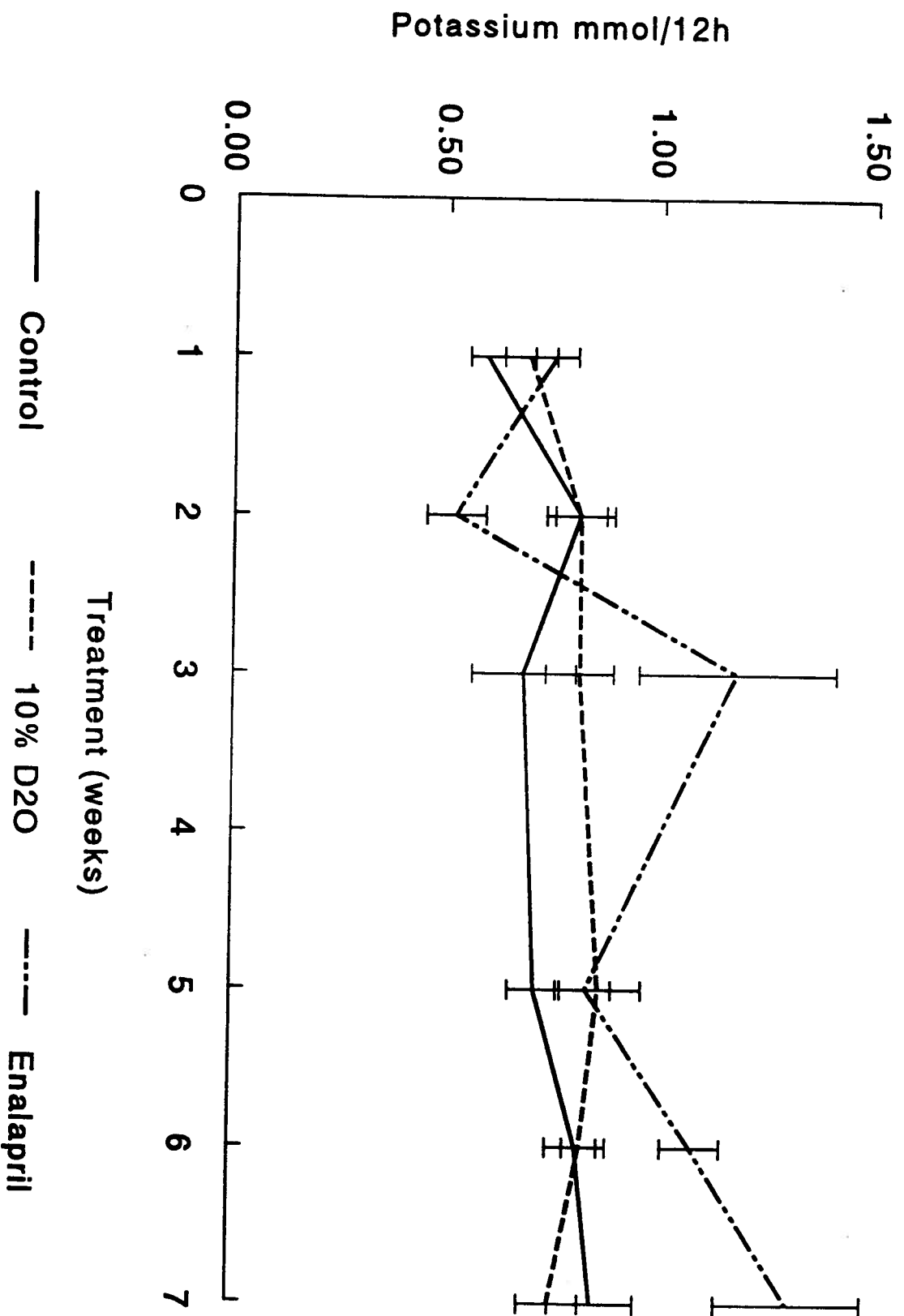
Figure 6. Urine Potassium

Figure 7. Urine sodium (mmol/12h) measured weekly multiplied by the 12 hour urine volume. There is no significant difference ($p > 0.05$) between the control and enalapril treated group. The 10% D_2O group has significantly lower sodium than both control and enalapril groups. Values are means ± S.E.M., n = 8 for each group. * indicates a significant difference from control and enalapril groups ($p < 0.05$).
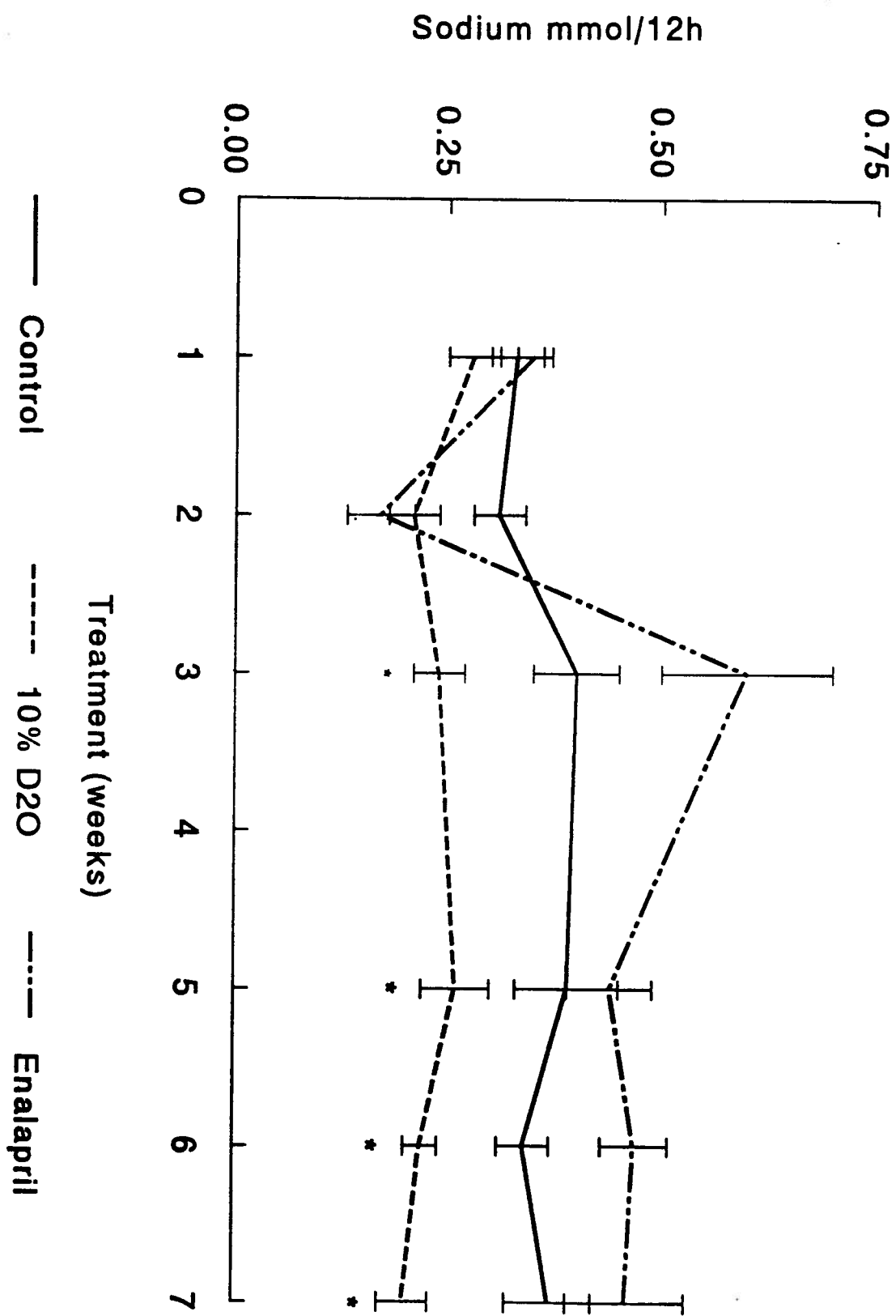
Figure 7. Urine Sodium

——— Control ----- 10% D2O ---·--- Enalapril

Sodium mmol/12h

0.75 0.50 0.25 0.00

Treatment (weeks)

0 1 2 3 4 5 6 7

Figure 8. Percentage of D_2O (v/v) in the urine measured weekly. There is an obvious difference between 10% D_2O (open circles) with control and enalapril (closed circles) where there was no detection of deuterium in the urine. The minimum level of detection with this method is 0.1%. Values are means ± S.E.M., n = 8 for each group.
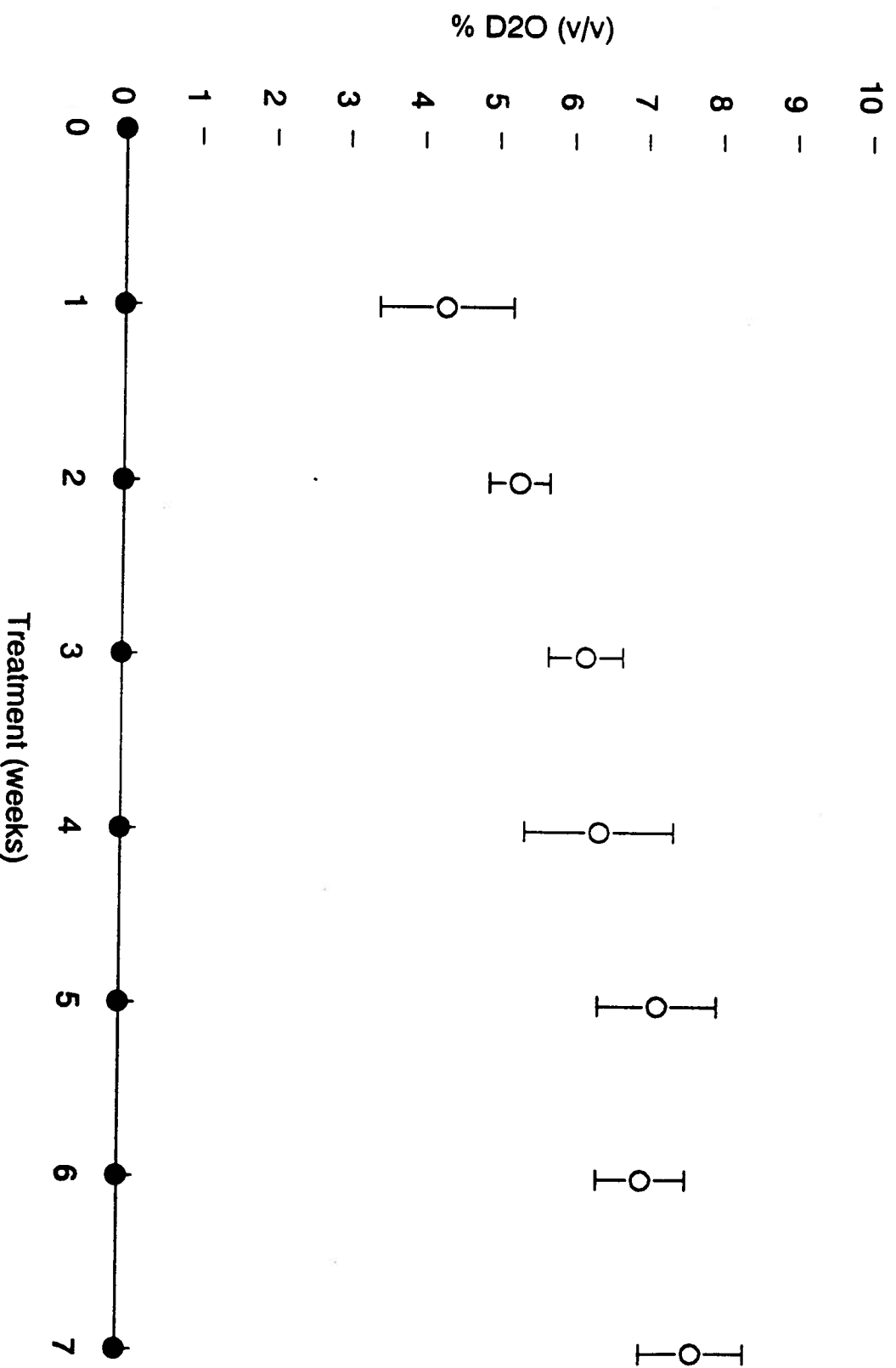
Figure 8. Percentage of D2O in Urine

Figure 9. Fasting plasma glucose concentrations (mmol/L) measured weekly
The group treated with 10% D_2O has significantly lower plasma glucose
concentrations than the enalapril treated group. There is no significant
difference between the control group and both treated groups. Values are
means ± S.E.M., n = 8 for each group. ** indicates a significant difference
between the 10% D_2O group and the enalapril group ($p < 0.05$).

Figure 9. Fasting Plasma Glucose

Figure 10. Fasting plasma triglyceride concentrations (mmol/L) measured weekly. Both treatment groups (10% D_2O and enalapril) have significantly lower triglyceride concentrations than the control group. . Values are means ± S.E.M., n = 8 for each group. * indicates a significant difference between the control and treatment groups ($p < 0.05$).

Figure 10. Fasting Plasma Triglycerides
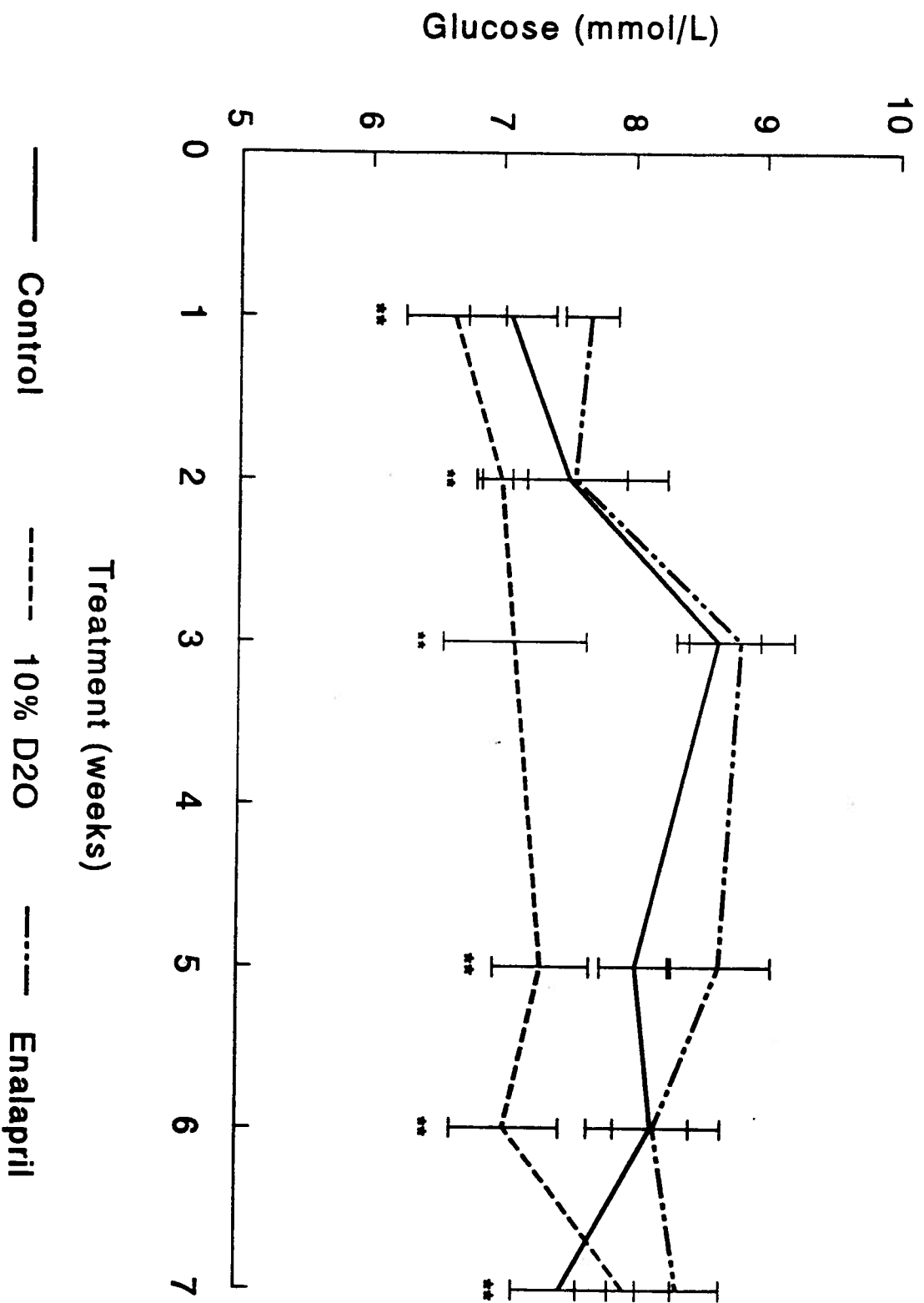
Triglycerides (mmol/L)

Treatment (weeks)

————— Control

- - - - - 10% D2O
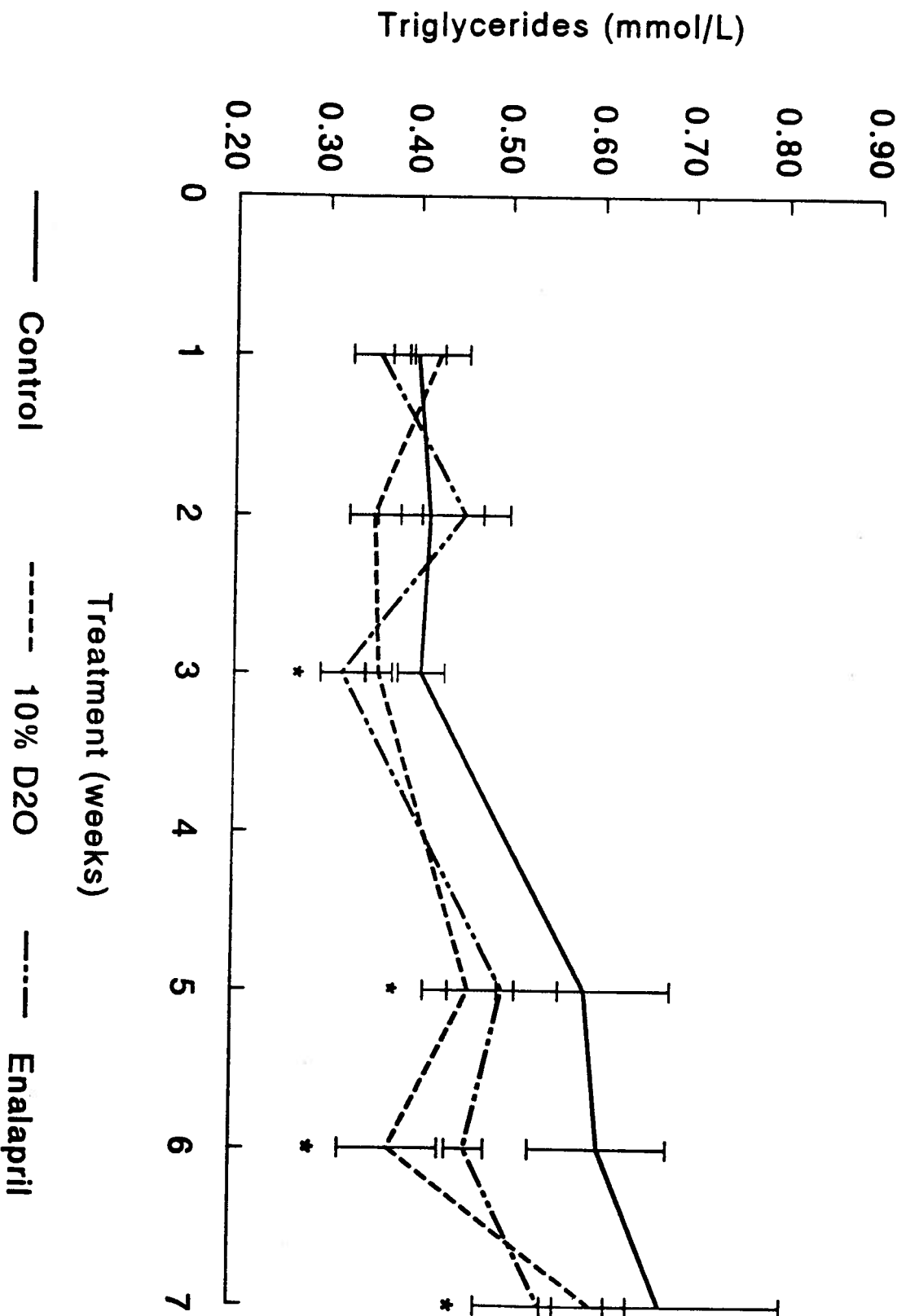
- · - · - Enalapril

Figure 11.   Fasting plasma insulin concentrations (mU/L) measured weekly. There is no significant difference in insulin concentration between the control and treatment groups ($p > 0.05$). Values are means ± S.E.M., $n = 8$ for each group.

Figure 11. Fasting Plasma Insulin

Figure 12.   Insulin:glucose ratio (mU:mmol) is representative of insulin resistance in the SHR.   There is no significant difference in the insulin:glucose ratio between the control and treatment groups. Values are means ± S.E.M., n = 8 for each group.

Figure 12. Insulin:Glucose Ratio

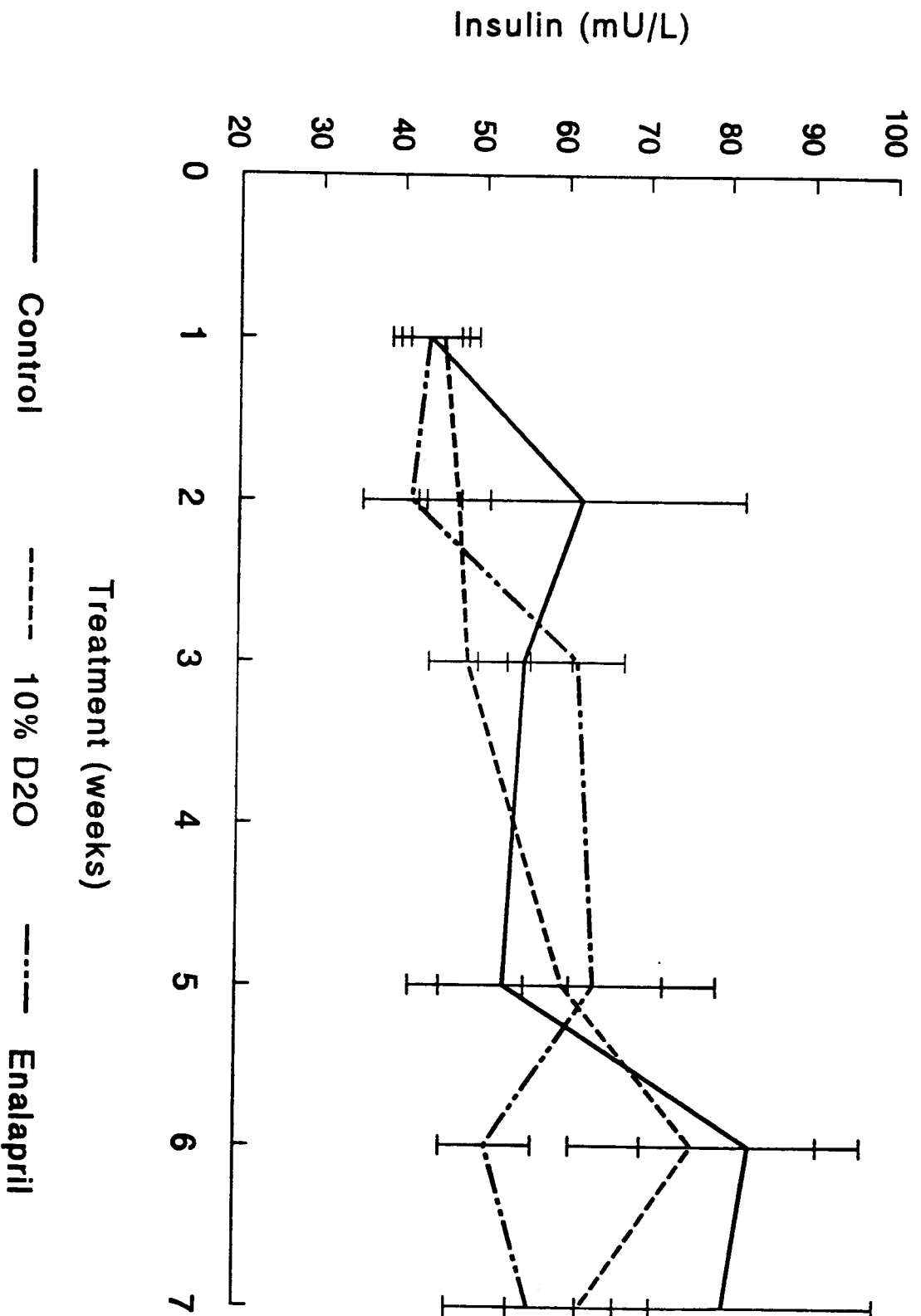Figure 13. Body weights (g) measured weekly. There was no significant difference between the control, 30, 100, and 300 mg/kg/day metformin treated groups (p > 0.05). Values are means ± S.E.M., n = 6 for each group.

Figure 13. Weekly Body Weights

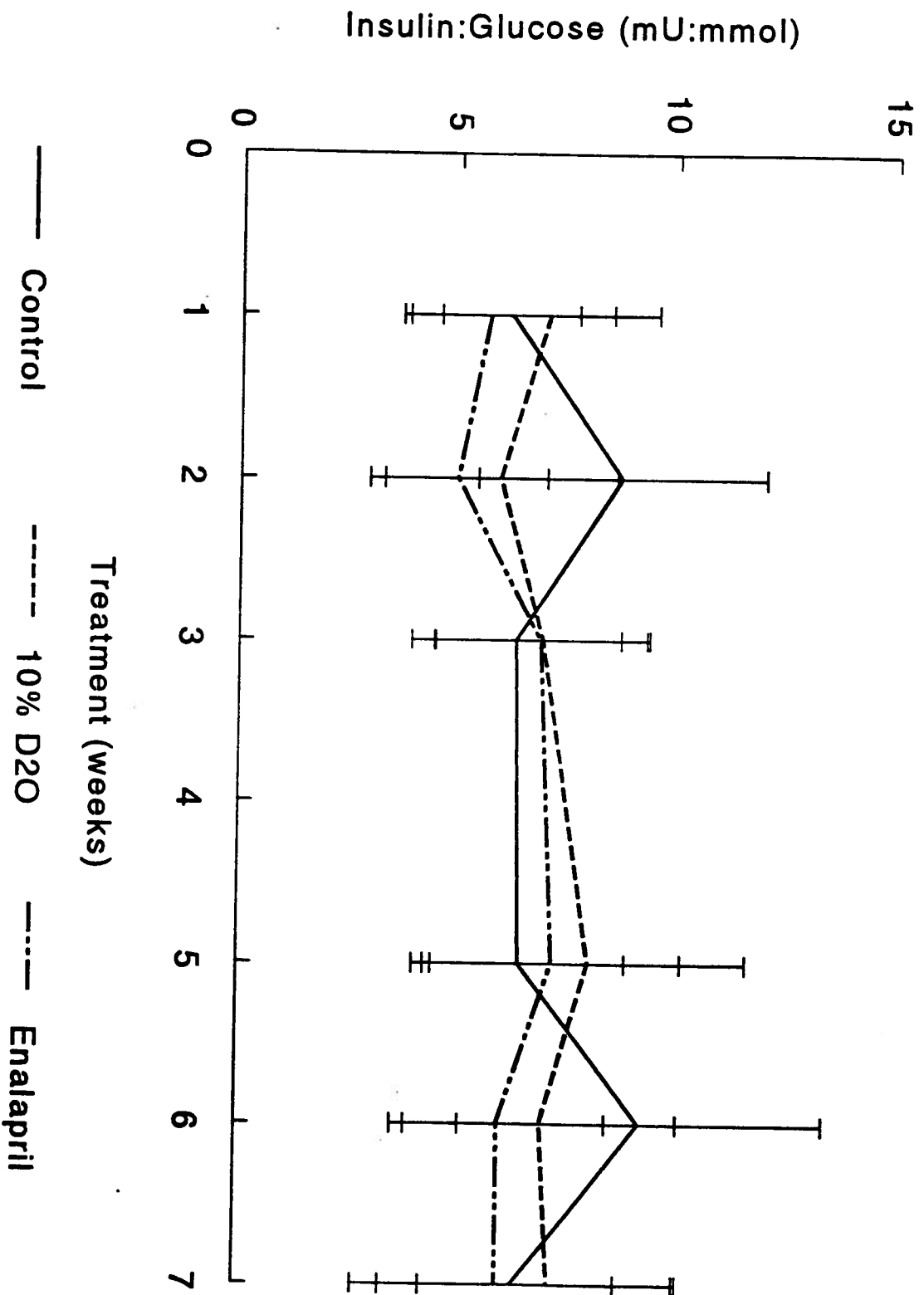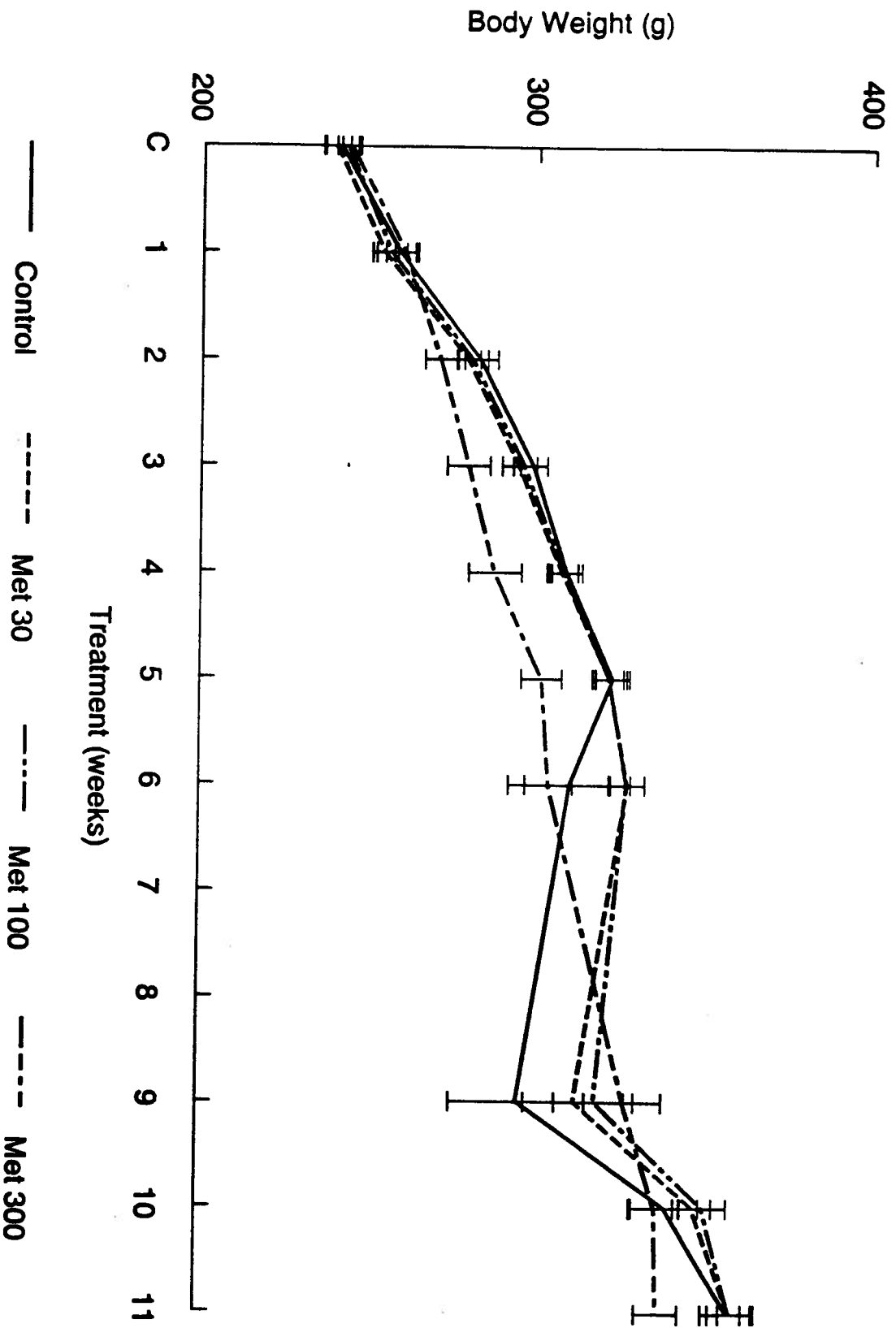Figure 14. Indirect systolic blood pressure (mmHg) measured weekly via tail cuff. There is no significant difference between the control, 30, 100, and 300 mg/kg/day metformin treated groups ($p > 0.05$). Values are means ± S.E.M., n = 6 for each group.

Figure 14. Systolic Blood Pressures
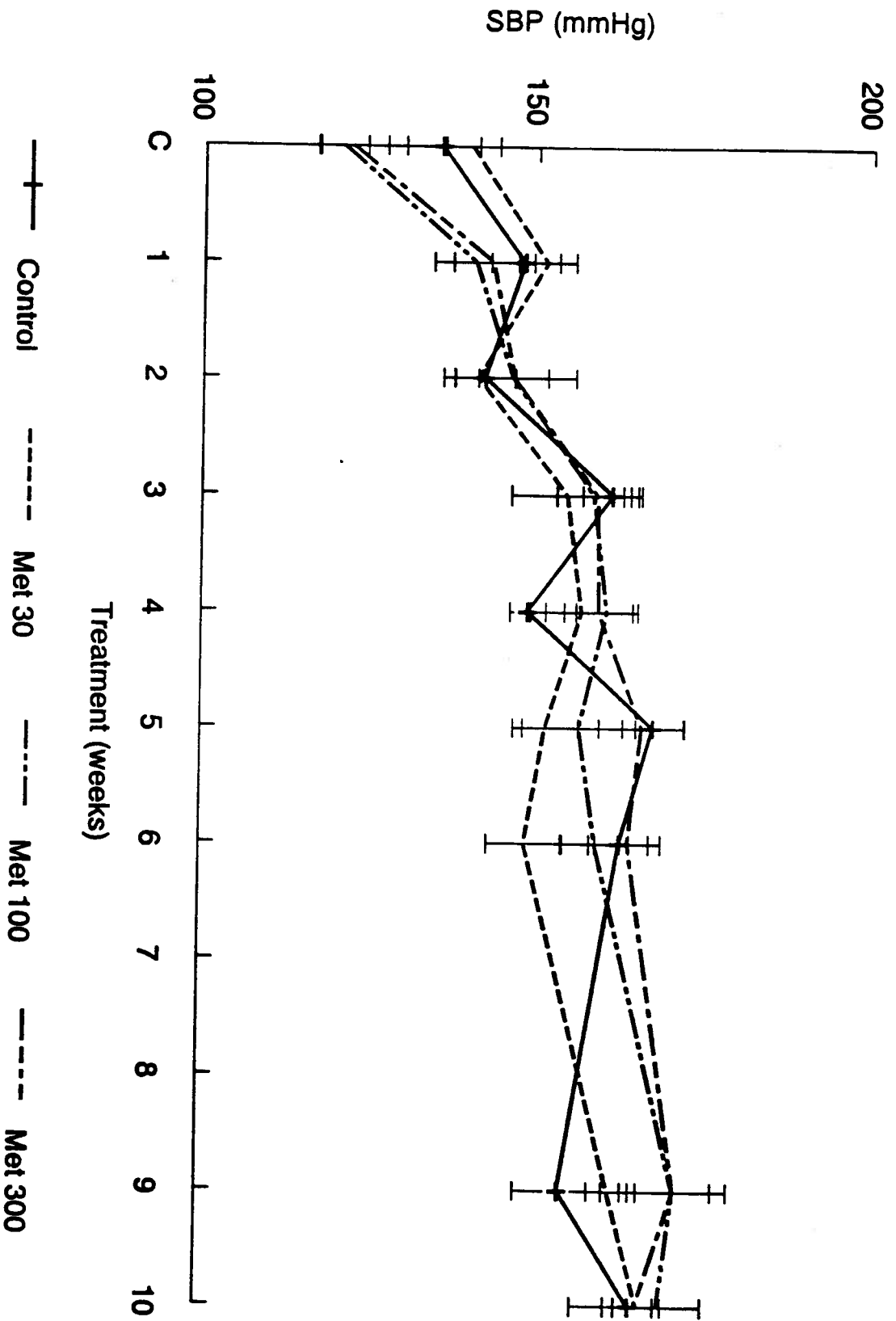
Figure 15. Direct blood pressure (mmHg) after ten weeks of treatment. There is no significant difference in systolic pressure (top of bar) between control, 30, and 100 mg/kg/day metformin treated groups, while the 300 mg/kg/day metformin treated group was significantly lower than the other groups (p < 0.05). The diastolic pressure (bottom of bar) of the 300 mg/kg/day metformin was also significantly lower than the other three groups. Values are means ± S.E.M., n = 6 for each group. * indicates significant difference in systolic pressure (p < 0.05). ** indicates significant difference in diastolic pressure.

Figure 15. Direct Blood Pressures
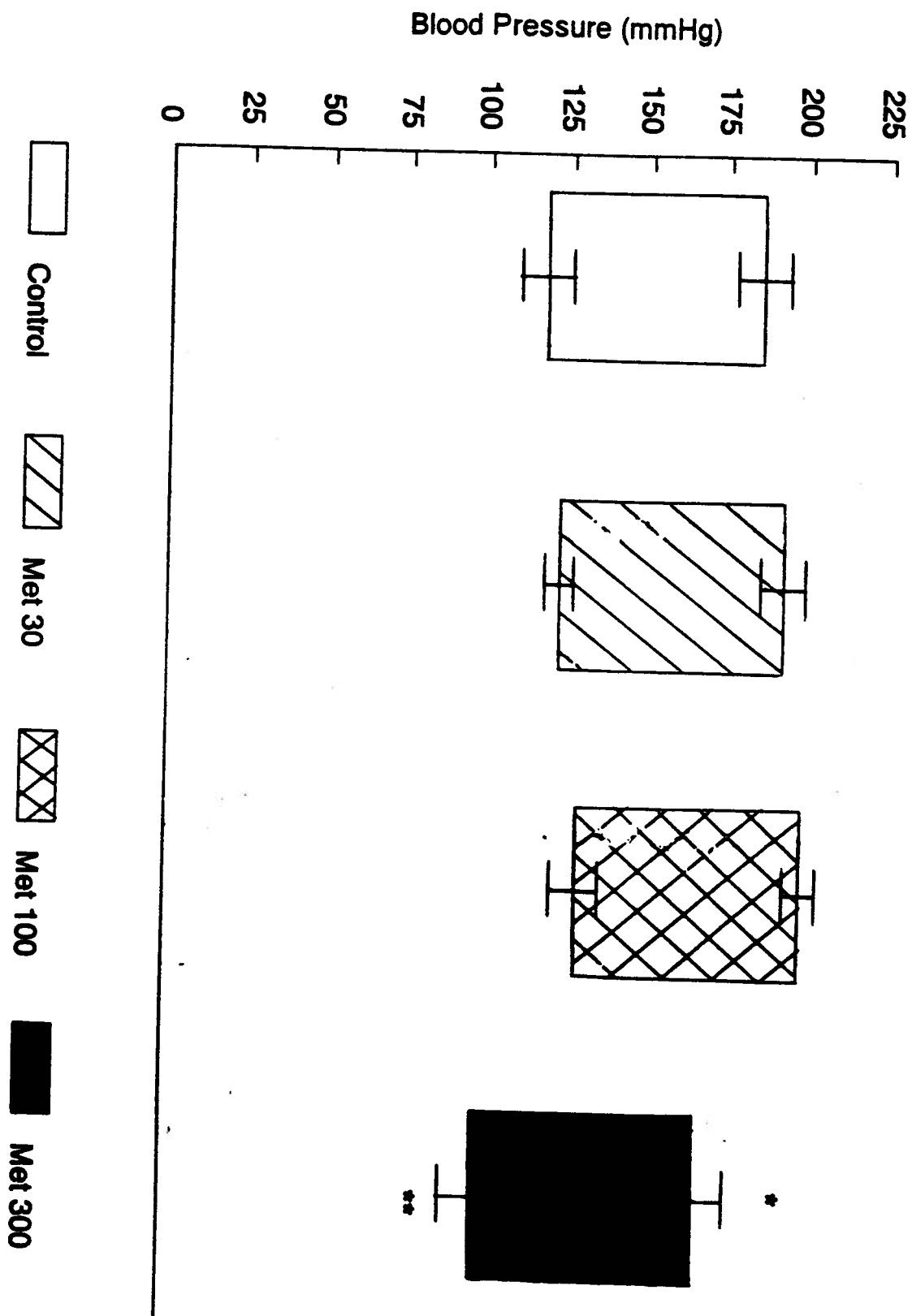
Figure 16. Twenty-four hour water intake (mL). No significant difference was found between the control and metformin treated groups. The 300 mg/kg/day metformin treated group had significantly lower water intake than the 30 mg/kg/day metformin treated group. Values are means ± S.E.M., n = 6 for each group. * indicates a signficant difference (p , 0.05) between the 30 and 300 mg/kg/day metformin treated groups.

Figure 16. 24 H Water Intake
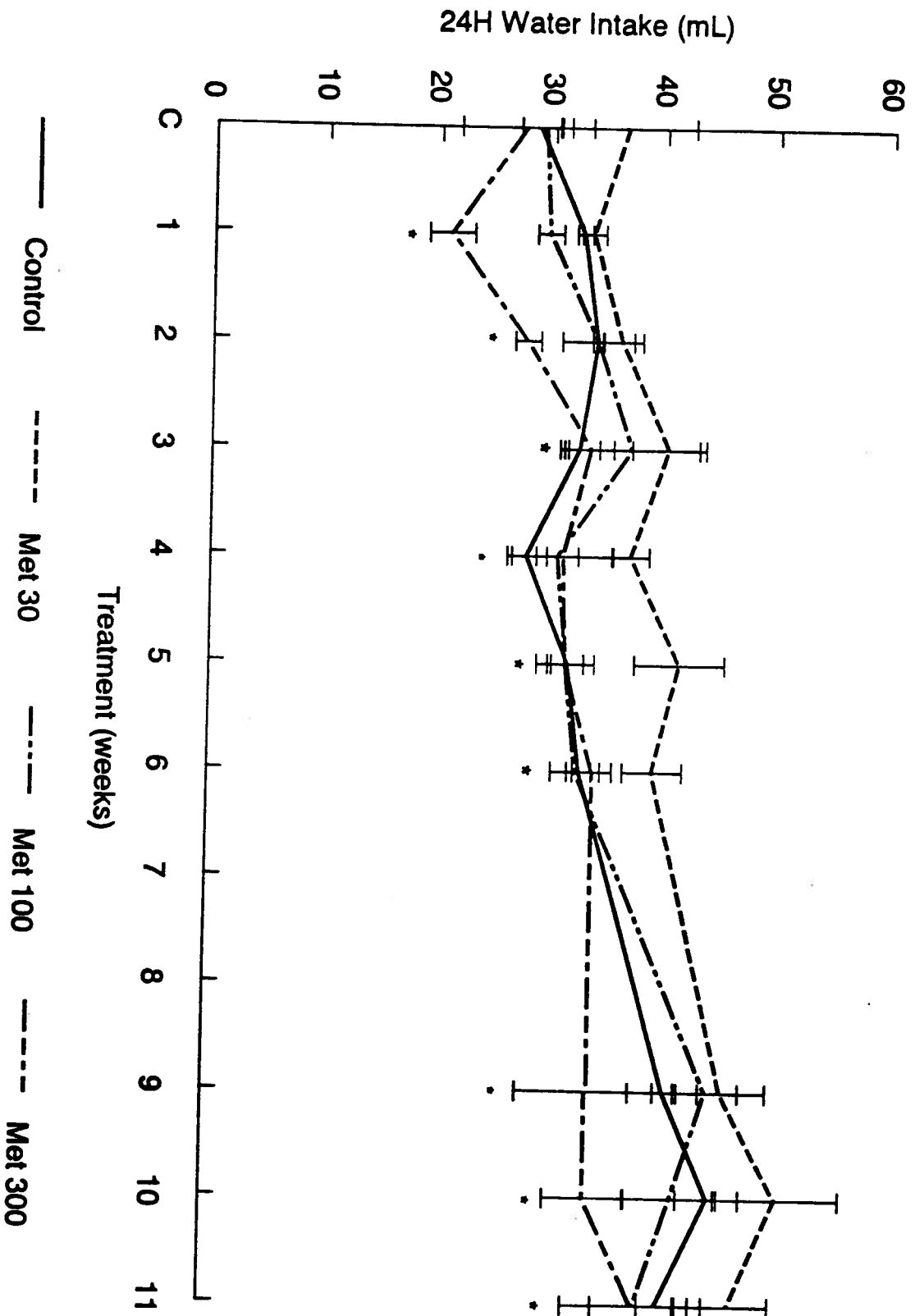
Figure 17. Weekly urine volumes (mL) measured over 24 hours. There was no significant difference between the control and treatment groups (p > 0.05). The 300 mg/kg/day metformin treated group had a significantly lower urine output than the 30 mg/kg/day metformin treated group over the treatment period. Values are means ± S.E.M., n = 6 for each group. * indicates a signficant difference (p , 0.05) between the 30 and 300 mg/kg/day metformin treated groups.

Figure 17. 24 H Urine Volume
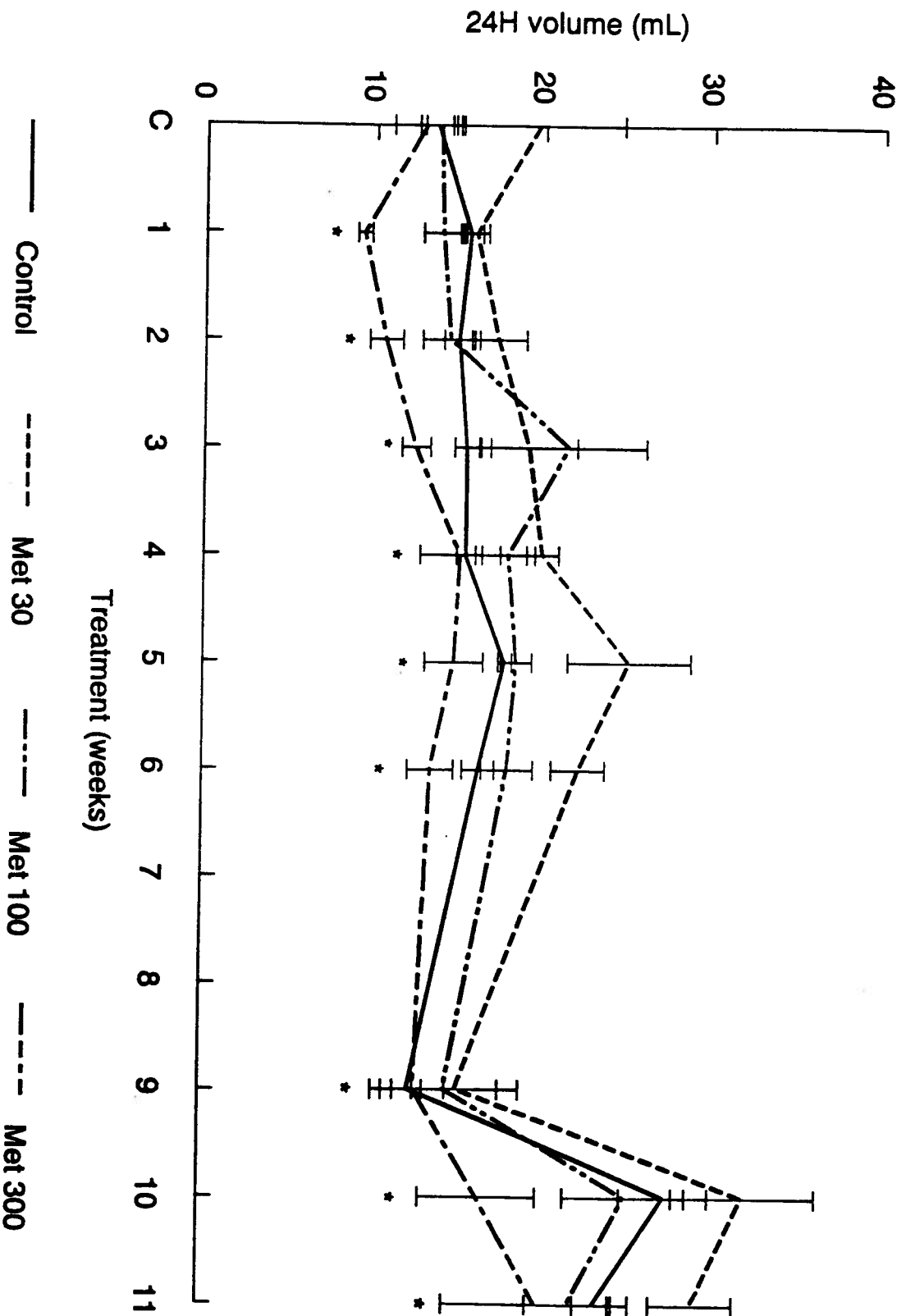
Figure 18. Fasting plasma glucose concentrations (mmol/L) measured weekly. There is no significant difference ($p > 0.05$) between the control group and the metformin treated groups, although there is a significant decrease in glucose concentration in all of the groups throughout the treatment period. Values are means ± S.E.M., n = 6 for each group.

Figure 18. Fasting Plasma Glucose
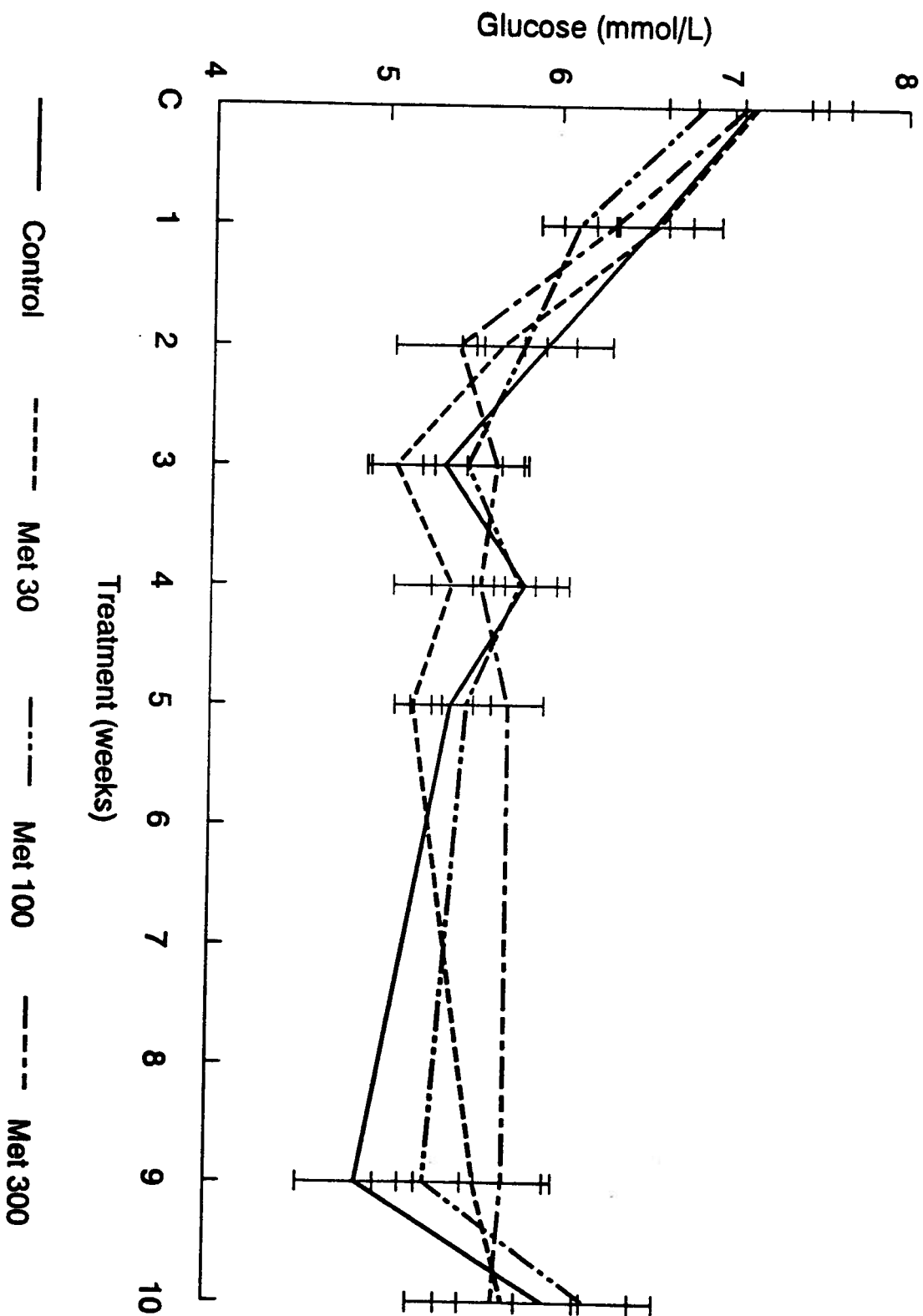
Figure 19.   Fasting plasma triglyceride concentrations (mmol/L) measured weekly.   No significant difference ($p > 0.05$) is observed between the control and treatment groups.   Values are means $\pm$ S.E.M., $n = 6$ for each group.

Figure 19. Fasting Plasma Triglycerides
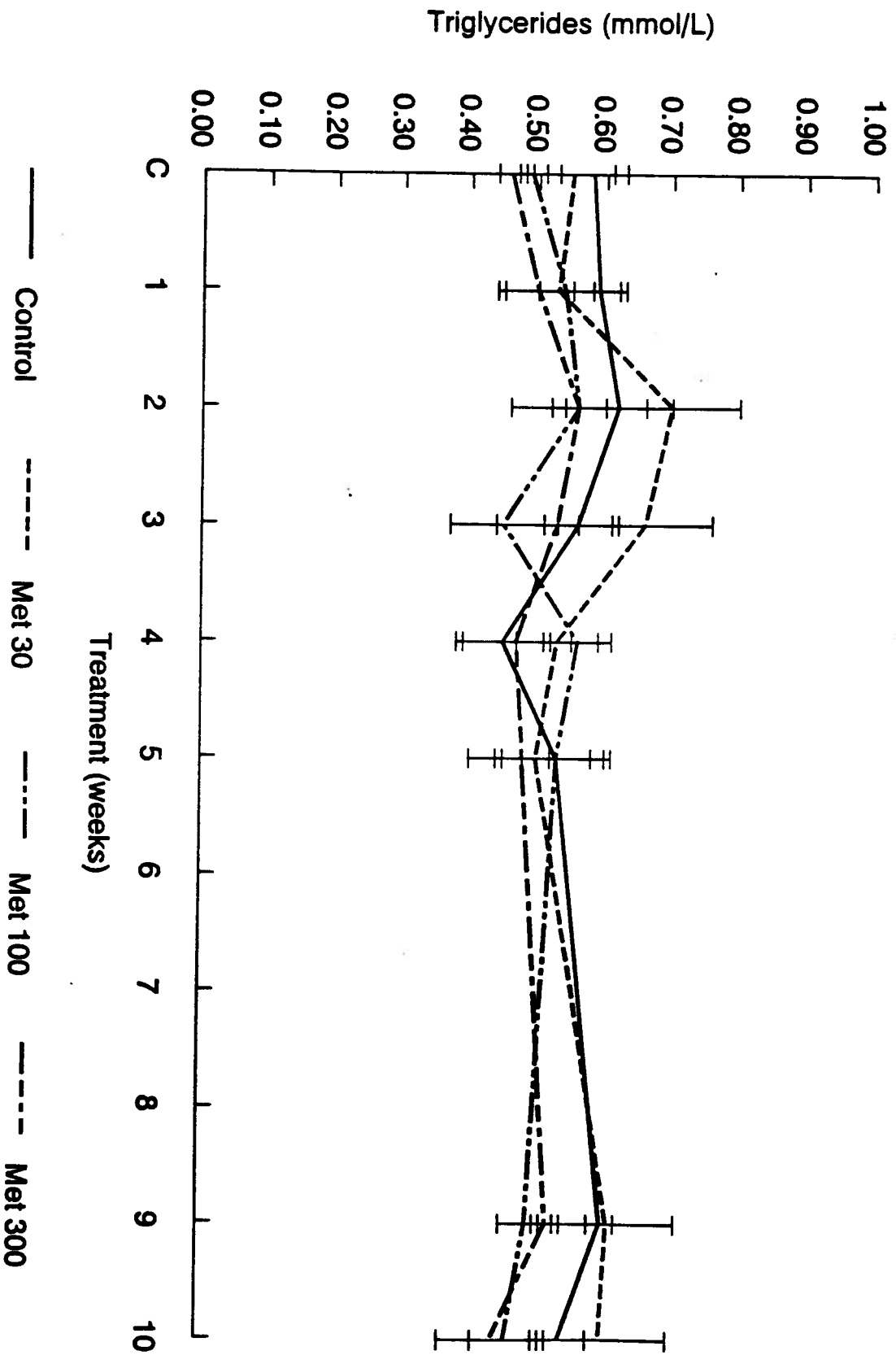
Figure 20. Fasting plasma insulin concentrations (mU/L) measured weekly. There is no significant difference ($p > 0.05$) in insulin concentrations between the control and treatment groups. Values are means ± S.E.M., n = 6 for each group.

Figure 20. Fasting Plasma Insulin
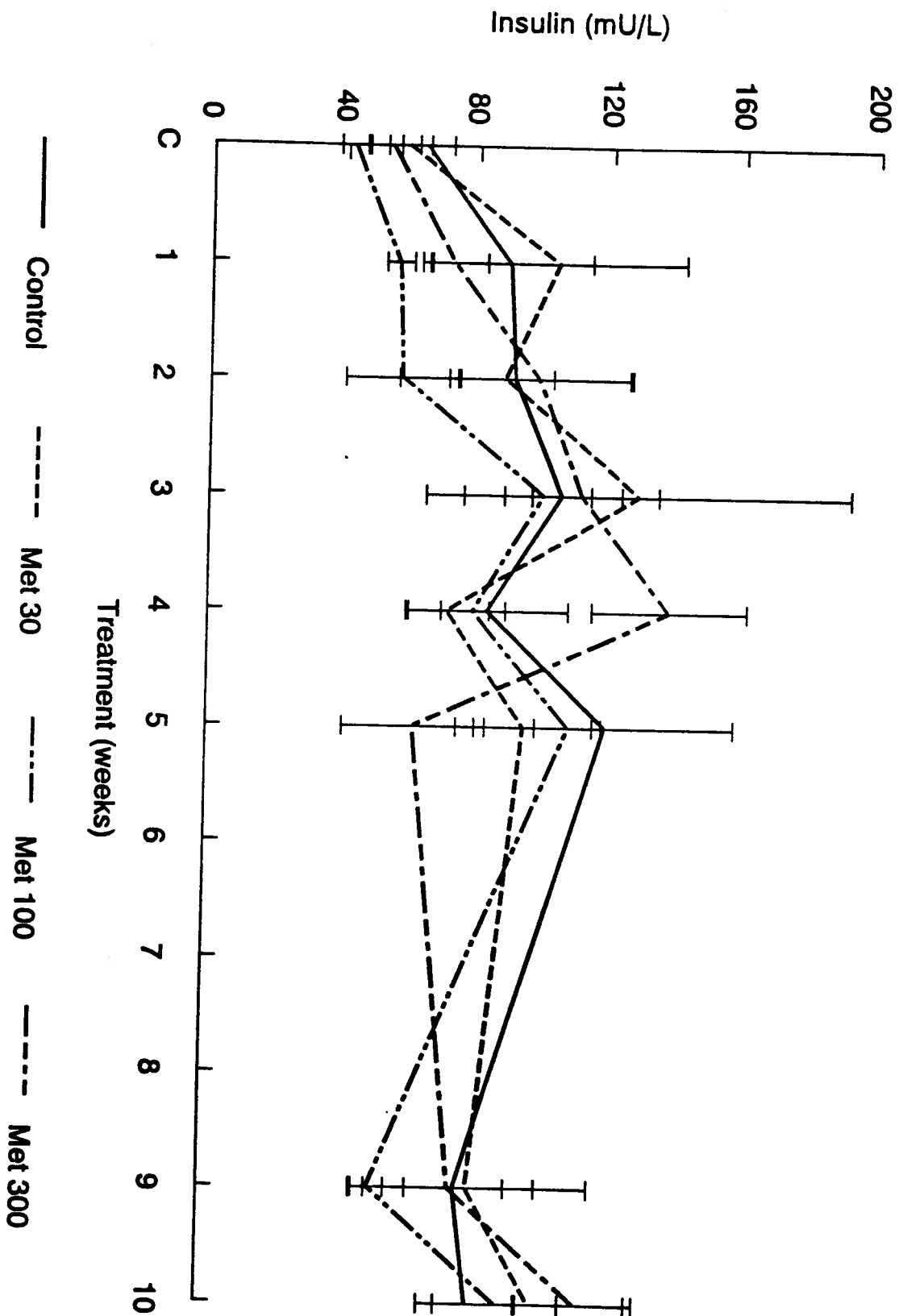
Figure 21. Insulin:Glucose ratio (mU/mmol) is representative of insulin resistance in the SHR. There is no significant difference ($p > 0.05$) in the insulin:glucose ratio between the control and treatment groups. Values are means ± S.E.M., n = 6 for each group.

Figure 21. Insulin:Glucose Ratio

Control ——

Met 30 - - - -

Met 100 — - —

Met 300 — - - —
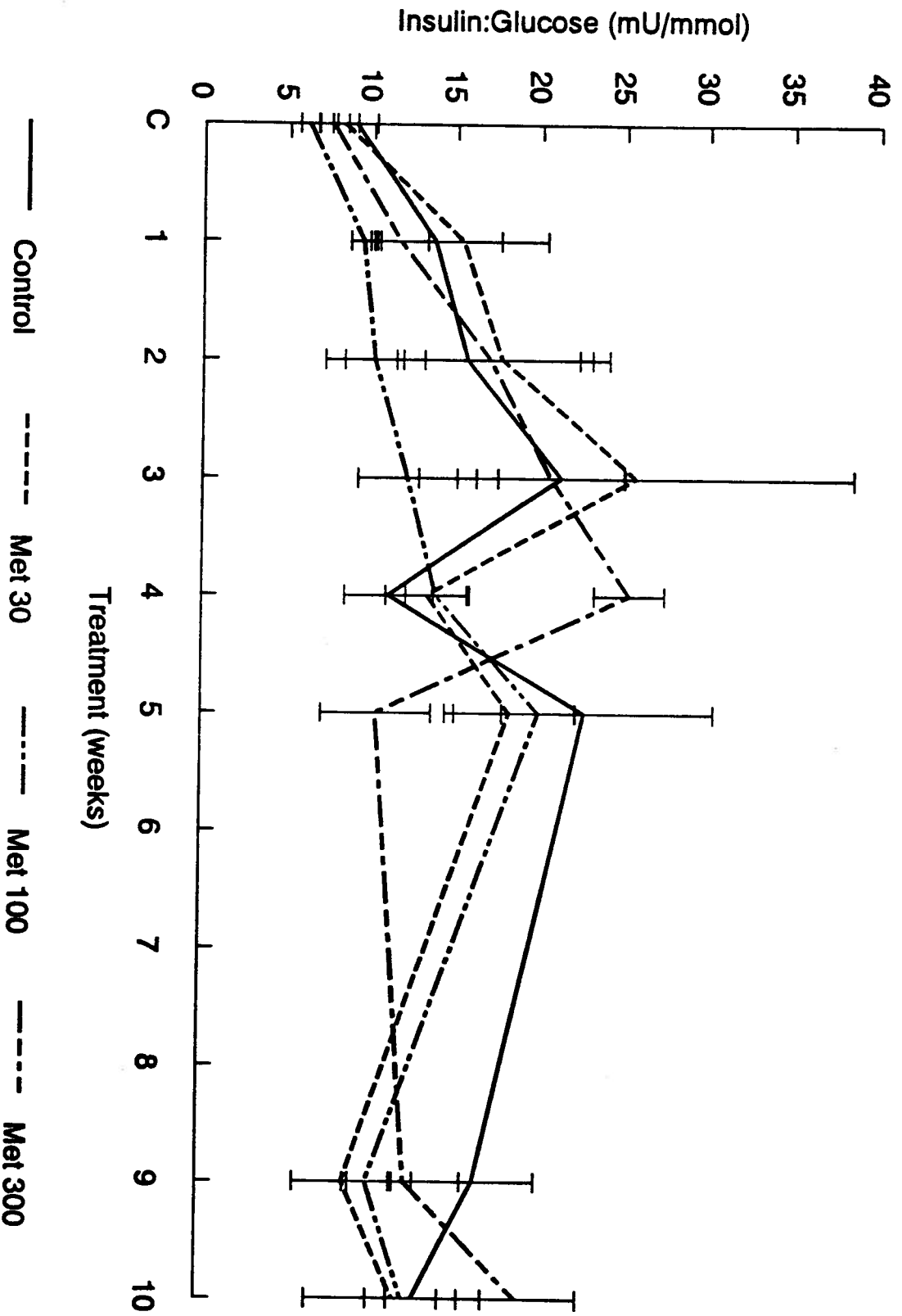
Table 1. Body weight, metabolic data, and blood pressure in the SHR at week 11 (final week) of metformin treatment.

Table 1. Body weight, metabolic data and blood pressures in the SHR at 11 weeks of metformin treatment

	Control (n = 6)	Metformin 30mg/kg/day (n = 6)	Metformin 100mg/kg/day (n = 6)	Metformin 300mg/kg/day (n = 6)
Body weight (g)	359 ± 7.8	359.7 ± 3.3	359.7 ± 6.4	337.7 ± 6.5 *
Fasting plasma insulin (mU/L)	24.2 ± 3.2	23.4 ± 2.9	20.1 ± 2.3	39.0 ± 14.7†
Fasting plasma glucose (mmol/L)	6.8 ± 0.4	7.1 ± 0.8	6.6 ± 0.7	9.0 ± 1.5†
Insulin:glucose ratio (mU/mmol)	3.5 ± 0.3	3.3 ± 0.3	3.1 ± 0.3	5.0 ± 2.5†
Fasting plasma triglyceride (mmol/L)	0.45 ± 0.05	0.34 ± 0.06	0.39 ± 0.03	0.45 ± 0.09
Systolic blood pressure (mmHg)	185.3 ± 8.3	192.3 ± 6.9	198.4 ± 5.1	166.3 ± 9.9†
Diastolic blood pressure (mmHg)	117.2 ± 7.8	122.0 ± 4.3	128.1 ± 7.6	97.7 ± 10.5†
24h Water intake (mL)	40.5 ± 1.6	47.0 ± 3.5	38.5 ± 3.8†	36.7 ± 6.8†
24h Urine volume (mL)	23.3 ± 1.2	29.2 ± 2.5	21.8 ± 2.4†	20.0 ± 5.5†

Mean values ± S.E.M. are shown

† significantly different from control (p < 0.05)

* significantly different from metformin 30 and metformin 100 (p < 0.05)

4. DISCUSSION

4.1. Deuterium oxide and Enalapril

Results from the deuterium oxide and enalapril study show that enalapril significantly lowers the systolic blood pressure (fig. 2) over the six week treatment period in the SHR. This was verified by the direct blood pressure measurement (fig. 3), which also showed a significant decrease in the systolic and diastolic blood pressure in the enalapril treated group compared to the control group. However, the insulin and glucose concentrations (fig. 11) were not significantly different from the control group, demonstrating that lowering the blood pressure in SHR does not have an appreciable effect on insulin resistance in this model.

The measurement of insulin and glucose concentrations has previosusly been done by euglycemic clamping in rats (25, 43). The clamping technique measures insulin and glucose concentrations over a short period in the concious rat (approximately 180 mins.). In our experiment we used repeated measures of insulin and glucose concentrations, throughout the treatment period (weekly). The euglycemic clamp is a more sensitive method than the repeated measures but with repeated measures the ability to detect small changes in insulin requirements should be increased over time.

The results above are in contrast earlier publications that suggest ACE-inhibitors improve insulin resistance in hypertensive patients (71, 72, 93). Hypertensive patients were treated with captopril and measured for insulin-promoted glucose uptake (71). Insulin and glucose concentrations were significantly reduced and insulin sensitivity was improved by 11% with captopril treatment. Similar results were seen in aged insulin-resistant hypertensive patients who were treated with five different ACE-inhibitors: captopril, enalapril, quinapril, ramipril, and lisinopril (93).

It has been suggested that the potassium sparing (hyperkalemia) effect of ACE-inhibitors may play a role in improving insulin sensitivity. Hyperkalemia has been implicated in patients with renal failure who are being treated with ACE-inhibitors, due to decreased renal potassium clearance via reduced aldosterone levels (74, 75). However, Scandling, et al. have shown that treatment with enalapril does not acutely impair extrarenal potassium homeostasis in men with normal renal function (76). It has also been suggested that ACE-inhibitors induce better overall potassium conservation under everyday life conditions (67). Increases in potassium concentration have been shown to potentiate insulin release from the pancreas in rats (77). Dietary-induced potassium deficiency reduced insulin secretion in response to sustained hyperglycemia in healthy subjects (78). Thus, the potassium sparing effect of ACE-inhibitors may exert a positive influence on glucose tolerance, in combination with their known antihypertensive actions.

Our results are supported by the view of Reaven and Chang, who suggest that hypertension per se does not cause insulin resistance in the SHR (94). Swislocki, et al. have also demonstrated that decreasing blood pressure in hypertensive patients does not necessarily affect abnormal insulin and glucose metabolism (95).

Santoro, et al. showed that chronic ACE-inhibition does not interfere with insulin's effect on glucose uptake (96), suggesting that ACE-inhibitors do not affect insulin resistance. Furthermore, in a comparison study between enalapril and captopril on insulin sensitivity in normotensive individuals, both ACE-inhibitors caused an increase in fasting insulin concentrations (97); the opposite of what one would expect if insulin resistance was decreased. Non-obese, non-insulin-resistant patients with mild-to-moderate hypertension, treated with enalapril, had significantly lower blood pressure but there was no effect on

glucose tolerance (98). This study was performed with Japanese patients which may only be indicative of ethnic differences, however, the results suggest that ACE-inhibitors may improve insulin sensitivity only in insulin-resistant patients.

Glucose intolerance or hyperglycemia is one of the abnormalities linked to Syndrome X. If hypertension directly causes insulin resistance we would expect to see a decrease in insulin and/or a decrease in glucose when the blood pressure is lowered. Insulin and glucose concentration, as a marker of insulin resistance, showed no significant difference between the three groups, suggesting that enalapril had no effect on the metabolic factors in the SHR (fig. 12). Therefore, we can conclude that 1) enalapril and D_2O do not have an effect on insulin metabolism or 2) the SHR does not have insulin or glucose abnormalities associated with elevated blood pressure.

Ten percent D_2O caused a decrease in fasting plasma glucose but no change in fasting plasma insulin. Despite this, the 10% D_2O group failed to show a significant decrease in systolic blood pressure when compared to the enalapril treated and control groups (fig 2). This was confirmed by direct blood pressure measurements which were not significantly different in the 10% D_2O treated group when compared to the control (fig. 3). One possible explanation for these observations is a decrease in insulin resistance. These results do not support a causal link between insulin resistance and blood pressure.

Our results are in contrast to the results published by Vasdev, et al. who demonstrated that 10% D_2O was effective in preventing the elevation of blood pressure in SHR (64). In both experiments the systolic blood pressure was measured indirectly by a tail cuff method and each pressure value was an average of 3-4 recordings. The only obvious difference was that in our experiments the blood pressures were measured by an observer who was blinded as to the treatment.

We confirmed that 10% D_2O was being administered to the D_2O treated group by measuring D_2O in the urine (fig. 8). The urine analysis, by infrared spectrometer, for D_2O showed an 8% recovery of D_2O, thus demonstrating that D_2O was being ingested and equilibrating with total body water in the rats. The fact that it didn't achieve 10% may be due to limitations in the method of detection and/or a contribution of water from food plus metabolism.

As well as having a significant decrease in systolic blood pressure the enalapril treated group displayed a significantly higher water intake than the control or 10% D_2O groups (fig. 4). Result reported by McLennan, et al. (99) demonstrated that SHR treated with 25 mg/kg/day of enalapril drank significantly more than the untreated controls. This increase in water intake due to chronic converting enzyme inhibition has also been reported by Ferrone, et al. (100). The significant increase in urine volume is consistent with the higher water intake observed in the enalapril group (fig. 5) but it is not clear which is the primary event. The increased water intake may be secondary to increased urine volume. Enalapril prevents the conversion of angiotensin I to angiotensin II, by inhibiting the angiotensin converting enzyme (101). Angiotensin II is the primary regulator of aldosterone secretion and aldosterone secretion is known to stimulate sodium and water reabsorption (101). Therefore, enalapril may prevent sodium and water reabsorption in the SHR by inhibiting aldosterone action. This lack of reabsorption may lead to a primary increase in urine volume followed by an increase in water intake to maintain water homeostasis in the body. The mechanism of this effect has yet to be elucidated.

The above experiment and other documented results (94) strongly suggest that lowering blood pressure in animals with hypertension and insulin resistance has no effect on elevated insulin concentrations and glucose intolerance. Similar results have been shown in human hypertensive patients

(95, 96). It is therefore unlikely that increased blood pressure causes insulin resistance. In order to test whether insulin resistance causes hypertension, SHR were treated with metformin.

4.2. Metformin Experiment

This second experiment examined if insulin resistance was the cause of hypertension by altering the blood glucose concentrations with metformin. Kaplan suggests that maneuvers that reduce hyperinsulinemia or improve insulin sensitivity may lower blood pressure (73).

The dosing range used in this experiment was chosen to cover the normal dose of metformin given to patients (30 mg/kg/day, 82, 102) as well as supermaximal doses (100 and 300 mg/kg/day) given to SHR in previous studies (92). Over the ten week treatment period the systolic blood pressure was not significantly lowered by any dose of metformin (fig. 14). These results are in contrast to Morgan, et al. who showed a significant decrease in mean arterial pressure in SHR treated with 200-250 mg/kg/day metformin, intraperitoneal (92). In both methods, the measurement of blood pressure was done when the rats were conscious. The difference occurred in the length of metformin treatment; Morgan's experiment was acute (7 days on metformin) whereas our experiment was chronic (10 weeks). It is interesting to note that hypertensive patients receiving 30 mg/kg/day metformin have a significant decrease in blood pressure over a 6 week treatment period (91, 102) but in our experiments doses of 30, 100, and 300 mg/kg/day metformin failed to reduce the blood pressure in the SHR. The reduction in direct blood pressure in the 300 mg/kg/day metformin treated group may be a manifestation of an interaction between the drug and the additional stress on the SHR due to the cannulation surgery prior to blood pressure measurement. It may also be due to an interaction between metformin and halothane because only 60-80% of absorbed halothane is eliminated

unchanged in the first twenty-four hours after inhalation (103). Therefore, the reduction in blood pressure seen in the higher doses of metformin is unlikely to represent a specific pharmacologic effect of the drug.

It has been shown that metformin enhances the basal rate of glucose transport (104) thus decreasing blood glucose concentrations in humans (91) at doses of approximately 30 mg/kg/day. Our results show no significant difference in fasting blood glucose (fig. 18), fasting blood insulin (fig. 20) concentrations, or insulin/glucose ratios (fig. 21) in the SHR over the dosing range. These results show that metformin has no effect on glucose homeostasis in this model. Explanations for this lack of effect include: 1) metformin does not have an effect on improving glucose concentration in the rat, 2) metformin does not have an effect in animals that do not express a diabetic state; i.e., SHR do not have insulin resistance, 3) our methods were not sensitive enough to detect an effect.

The final blood samples taken at week 11 show significantly higher fasting glucose and insulin concentrations in the 300 mg/kg/day metformin group compared to the control group. This effect is opposite to the expected effect of metformin, as discussed above. One of the reasons for this discrepancy may be due to the added effect of the high dose of the drug plus the invasive procedure performed on the rats prior to blood sampling. Rao suggests that stress from blood loss may be a source of error in the evaluation of glucose turnover and insulin sensitivity (105). Another explanation may be an interaction of the drug and anesthetic on glucose and insulin concentrations. It can be concluded that the elevated concentrations of glucose and insulin in the final plasma samples are most likely toxicological effects of the metformin treatment causing stress, interacting with the invasive procedure or anesthetic agent.

4.3. Is the SHR an Effective Model of Insulin Resistance?

Although we failed to show an effect on insulin resistance as a result of blood pressure reduction and increased insulin sensitivity in the SHR, there is conflicting evidence as to whether this model accurately represents the insulin resistant state as seen in human patients (45, 46). As previously mentioned, the SHR has been described as an appropriate experimental model for both hypertension and insulin resistance (41, 42, 43). These studies suggest a link between high blood pressure and high insulin levels in this rat model. However, Horl, et al. showed no evidence of peripheral insulin resistance between SHR and WKY rats from the response of skeletal muscle to insulin. After a 12h fasting period, muscle glucagon and glucose levels were almost identical for the two groups of rats (46). These results suggest no difference between SHR and its control strain, which further suggests that the SHR is not insulin resistant because the WKY does not display insulin resistance. Furthermore, Buchanan, et al. used glucose clamp studies to show that insulin stimulated glucose uptake was not different between age-matched SHR and WKY rats (45). This evidence was seen in the SHR when their systolic blood pressure was significantly elevated, compared to the WKY. Therefore, the SHR may not be an effective model for studying the relationship between hypertension and insulin resistance as seen in human patients.

It is of interest to note that there are also inconsistencies in the association between insulin resistance and hypertension in human subjects. O'Brien, et al. looked at patients with insulinoma to determine if hyperinsulinemia contributed to the pathogenesis of hypertension in the absence of insulin resistance (106). They showed no significant difference in systolic and diastolic blood pressure between the control patients and patients with insulinoma. The prevalence of hypertension was also similar between patients with insulinoma

and matched controls. It was concluded that hyperinsulinemia could not be implicated in the genesis of hypertension.

4.4. Conclusions

1. Ten % D_2O decreases fasting plasma glucose concentrations, thus possible causing a decrease in insulin resistance.

2. Despite this, chronic 10% D_2O has no effect on blood pressure or fasting plasma insulin concentration in the SHR.

3. This suggests that insulin resistance does not cause increased blood pressure.

4. Enalapril decreases blood·pressure but has no effects on glucose and insulin concentrations in the SHR, confirming that high blood pressure does not cause insulin resistance.

5. Enalapril causes a large increase in urine volume and water in the SHR.

6. Chronic metformin (10 to 300 mg/kg/day) has no effect on insulin and glucose concentrations or blood pressure in SHR.

7. The SHR may not be an appropriate model for studying the link between hypertension and insulin resistance.

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