JPET Fast Forward. Published on October 13, 2003 as DOI: 10.1124/jpet.103.054221
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JPET Fast Forward. Published on October 13, 2003 as DOI:10.1124/jpet.103.054221

CONTRASTING METABOLIC EFFECTS OF ANTIHYPERTENSIVE AGENTS[1]*

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Running Title: ANTIHYPERTENSIVES & METABOLISM

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Text Pages: 32

Number of Tables: 1

Number of Figures: 6

Number of References: 38

Abstract: **247 words**

Introduction: **791 words**

Discussion: **1639 words**

Sections: Endocrine & Reproductive

Abbreviations: α_2AR: α_2-adrenergic receptor; AUC: area under the curve; **FFA: plasma free fatty acids; I_1R: I_1-imidazoline receptor; SHROB: spontaneously hypertensive rat, obese substrain**

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ABSTRACTHypertension often co-exists with hyperlipidemia, insulin resistance and glucose intolerance, a comorbidity known as metabolic Syndrome X. Different antihypertensives have mixed effects on these associated abnormalities. We compared three antihypertensives in the spontaneously hypertensive obese rat (SHROB) model of Syndrome X. Moxonidine (4 mg/kg), an imidazoline and α_2-adrenergic agonist, α–methyldopa (200 mg/kg), an α_2-adrenergic agonist, or the vasodilator hydralazine (10 mg/kg) were given orally for 15d. All three agents lowered blood pressure equally. Moxonidine significantly reduced fasting plasma insulin, glucagon, cholesterol, triglycerides and free fatty acids (FFA) compared to untreated controls. In contrast, Syndrome X markers were not affected by α-methyldopa treatment, and hydralazine reduced only glucagon and FFA. Relative to untreated controls, moxonidine improved glucose tolerance as shown by reduced glucose area under the curve (AUC) (13.6 ± 2.4 vs. 42.5 ± 9.9 g*min/dL). Insulin AUC was increased (7.4 ± 0.9 vs. 3.9 ± 1.8 µg*min/mL) as was the plasma C-peptide response to the glucose load. In contrast, α-methyldopa and hydralazine worsened glucose tolerance (68 ± 26 and 110 ± 21 g*min/mL, respectively) and significantly reduced insulin AUC (2.5 ± 0.8 and –2.3 ± 1.0 µg*min/mL, respectively) compared to controls. Moxonidine reduced but α-methyldopa and hydralazine elevated glucagon levels after the glucose load. Contrary to the "hemodynamic hypothesis" for the metabolic actions of antihypertensives, which predicts roughly equal benefits, only moxonidine had a positive impact on comorbidities. This unique action suggests a role for direct stimulation of imidazoline receptors.

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Many therapeutic agents have established efficacy in hypertension. However, hypertension rarely occurs in isolation. Metabolic syndrome X is a cluster of metabolic diseases including hypertension, insulin resistance, hyperlipidemia, glucose intolerance and obesity. This syndrome frequently precedes the development of type II diabetes and atherosclerosis. The obese spontaneously hypertensive rat (SHROB; Koletsky rat) is a unique animal model of metabolic Syndrome X with genetic obesity superimposed on a background of genetic hypertension (Koletsky et al., 2001). The obese phenotype results from a nonsense mutation in the leptin receptor gene, designated *fa*^{k}, which is a naturally occurring knockout of all forms of the leptin receptor (Takaya et al., 1996). The *fa*^{k} mutation imposed on a hypertensive background results in extreme hyperinsulinemia, hyperlipidemia, glucose intolerance and decreased expression of insulin signaling proteins in skeletal muscle and liver (Friedman et al., 1997).

Antihypertensive agents differ in their impact on glucose and lipid homeostasis. Human studies are not unanimous, but in general thiazide diuretics and β-adrenergic antagonists have slight adverse effects, calcium channel blockers are mixed, and α_1-antagonists and inhibitors of the renin-angiotensin system have positive effects (Imazu, 2002;Rabbia et al., 2001). Several theories have been advanced to account for the metabolic effects of various classes of antihypertensives. A prevalent theory that could be called the “hemodynamic hypothesis” postulates that substrate and hormone delivery to target tissues is a major limitation on glucose disposal in hypertension (Julius et al., 1992). Thus, antihypertensives with direct or indirect vasodilating actions will improve glucose disposal, whereas agents such as β-blockers that reduce cardiac output and increase vascular resistance will worsen glucose disposal. The supporting evidence for

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this hypothesis comes from clinical drug trials and tests of human forearm microcirculation but few laboratory trials have been carried out.

A related hypothesis postulates a central role for the sympathoadrenal system in the integration of cardiovascular and metabolic actions of drugs (Julius et al., 1992;Ernsberger et al., 1998) . In addition to possible hemodynamic effects, sympathoadrenal activation inhibits insulin secretion and promotes glucagon secretion from the pancreas, activates glycogenolysis and gluconeogenesis and elevates FFA among other possibly adverse effects (Ernsberger et al., 1998). Thus, sympatholytic agents such as α_1-antagonists and centrally acting antihypertensives may have beneficial metabolic effects. Conversely, antihypertensive agents that evoke reflex sympathoexcitation may have adverse metabolic effects (Jamerson et al., 1993). The beneficial effects of α_1-adrenergic antagonists on both glucose and lipid metabolism have been extensively studied in humans and animals, and several recent studies indicate that central sympatholytic agents of the imidazoline class improve glucose metabolism in humans and in animal models (Esler et al., 2001;Bauduceau et al., 2000;De Luca et al., 2000;Ernsberger et al., 1996; 1999;Haenni and Lithell, 1999;Yakubu-Madus et al., 1999;Henriksen et al., 1997;Rosen et al., 1997).

The prototypical imidazoline central sympatholytic agents are moxonidine and rilmenidine (Chan and Head, 1996). They are agonists at the I_1 imidazoline receptor (I_1R) as well as α_2AR and act in the medulla oblongata to inhibit the sympathoadrenal system and lower blood pressure. Moxonidine's affinity at I_1R affinity is 40 times greater than at α_2AR (Ernsberger et al., 1993), but a contribution of α_2AR to its actions cannot be ruled out (Szabo et al., 2001). Clinical studies suggest that treatment of hypertension

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with the antihypertensive agent moxonidine may lower glucose levels in hyperglycemic patients (Haenni and Lithell, 1999). Studies in several experimental models, including spontaneous hypertensive obese rats (SHROB) and lean spontaneous hypertensive rats (SHR) (Ernsberger et al., 1999;Ernsberger et al., 1996) and in fructose-fed hypertensive rats (Rosen et al., 1997) all showed that chronic moxonidine therapy improves glucose tolerance and the insulin response to a glucose load. Indeed, chronic moxonidine therapy has been shown to enhance skeletal muscle glucose transport in insulin-resistant obese Zucker rats in vitro (Henriksen et al., 1997). Chronic moxonidine treatment also improved glucose homeostasis in Zucker diabetic fatty rats (Yakubu-Madus et al., 1999). None of these studies examined the question of which receptor(s), I_1R, α_2AR or both, mediate these metabolic effects, but it has been hypothesized that I_1R located either in the brainstem autonomic centers or in the periphery are responsible (Ernsberger et al., 1999).

In contrast to the "I_1R hypothesis", it has been proposed that all of the therapeutic actions of moxonidine and other imidazolines can be entirely accounted for by their activity at α_2AR (Szabo et al., 2001;Zhu et al., 1999). This conclusion is based almost entirely on studies of centrally mediated cardiovascular responses. Since both I_1R and α_2AR can mediate comparable sympathoinhibitory responses, separation of their actions is difficult, even using mutant mouse models (Zhu et al., 1999;Tolentino-Silva et al., 2000). In the present study, we examined metabolic responses to I_1R and α_2AR agonists, given the possibility that responses to these two classes of receptor may not be identical in all respects.

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The present study was thus designed as an indirect test of four competing hypotheses regarding the metabolic effects of antihypertensives. Each hypothesis makes specific predictions regarding the effectiveness of the three comparison agents. According to the hemodynamic hypothesis, all three agents should improve glucose and lipid metabolism by decreasing peripheral vascular resistance. The sympathoadrenal hypothesis predicts beneficial effects of the sympathoinhibitory agents moxonidine and α-methyldopa, whereas hydralazine, by eliciting reflex sympathoexcitation, should impair metabolic homeostasis. The I_1R hypothesis predicts unique beneficial effects of the I_1R agonist moxonidine. The α_2AR hypothesis predicts identical responses to treatment with the two α_2AR agonists, moxonidine and α-methyldopa, especially since the active metabolite of α-methyldopa, α-methylnorepinephrine, has nearly identical affinity for α_2AR as moxonidine (Ernsberger et al., 1993). Thus, we compared the effects of moxonidine, α-methyldopa and hydralazine on blood pressure and circulating glucose, insulin, insulin C-peptide, glucagon, triglycerides, cholesterol and free fatty acids in the fasted state and following a glucose load.

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Methods

Materials

Moxonidine was provided by Solvay Pharmaceuticals (Hannover, Germany). α-methyldopa and hydralazine were obtained from Sigma-Aldrich (St. Louis, MO).

Animal Procedures

Adult male and female obese spontaneously hypertensive rat (SHROB) were used in these studies. Animals were housed individually and were provided food (Tek lad 8664) and water ad libitum prior to drug treatment. Animals were on a 12:12-h light-dark cycle (lights on from 7:00 to 19:00) and were maintained at a constant temperature of 21ºC. These procedures were carried out with the approval of the Case Western Reserve University Animal Care and Use Committee.

Chronic Drug Treatment

All drugs were dissolved in 0.1% citric acid. SHROB were administrated drugs orally for 15d by admixture with powdered rat chow (identical formulation to standard chow) prior to pelleting. Food intake was allowed ad libitum and monitored continuously so that adjustments could be made to drug concentrations as necessary. Moxonidine, a mixed I_1R/α_2AR agonist, was administrated at 4 mg/kg/d. Preliminary studies indicated that this dose was as effective as the previously used 8 mg/kg/d (Ernsberger et al., 1996) α-Methyldopa, an α_2AR agonist, was given at a dose of 200 mg/kg/d shown to be effective in SHR (Tabei et al., 1970). Hydralazine, a direct vasodilator, was given at a dose of 10 mg/kg/d, which had previously been shown to control hypertension in the SHR model (Sabbatini et al., 2001). Control untreated SHROB were fed normal chow. Body weight and food intake were determined every other day. After 12d of treatment,

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tail cuff blood pressure was determined between 12:00 and 16:00 following habituation of the animals to the testing procedure. Systolic blood pressures were averaged between 3 and 5 measurements in a single session separate from the habituation sessions. After 15d of treatment, an oral glucose tolerance test (OGTT) (6 g/kg glucose by oral gavage) was conducted after an 18h fast.

Oral Glucose Tolerance Test (OGTT)

As previously described (Ernsberger et al., 1999), rats were given by oral gavage a 50% glucose solution at a dose of 6g/kg body weight. Blood (0.2 ml) was obtained from the tail of conscious animals at baseline and 30, 60, 120, 240 and 360 min after the glucose load. Plasma glucose, insulin and C-peptide were determined at each time point. Plasma glucagon and free fatty acids (FFA) were determined at 0, 30 and 60 min only. Area under the curve (AUC) was determined for each parameter for quantification of the OGTT.

Plasma Biochemical Measurements

Blood samples were chilled on ice, centrifuged for 20 min at 5000g at 4ºC, and the plasma frozen at –70ºC until assayed for plasma glucose, insulin, C-peptide, glucagon, triglyceride, total cholesterol and FFA. Plasma glucose was determined by colorimetric glucose oxidase assay (Sigma-Aldrich, St. Louis, MO). Plasma insulin, C-peptide and glucagon radioimmunoassay kits were used with rat insulin, C-peptide and glucagon standards and antibodies directed against rat insulin, C-peptide and glucagon, respectively (Linco, St Charles, MO). Plasma triglycerides and total cholesterol were determined by a colorimetric, enzymatic assay (Sigma-Aldrich, St. Louis, MO). Plasma FFA were determined by a colorimetric, enzymatic kit (Wako Chemicals, Richmond,

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VA). Assays were conducted in duplicate. Intra-assay coefficient of variation was 0.8% for glucose, 1.1% for glucagon, 2.9% for C-peptide, 3.7% for insulin, 2.5% for total cholesterol, 2.4% for triglyceride and 4.8% for FFA. The inter-assay variation was 2.3% for glucose, 7.2% for glucagon, and 6.2% for both C-peptide and insulin, 5% for total cholesterol, 3.7% for triglyceride and 7.2% for FFA.

Statistical Methods

Results are presented as means ± standard error of the mean. Comparisons between groups were made using one- or two-way analysis of variance (ANOVA) or analysis of variance with repeated measures (REMANOVA) using Prism (Graph Pad Software, San Diego, CA) with post-hoc analyses by Neuman-Keuls test.

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Results

Food Intake, body weight and blood pressure

Average food intake did not differ between groups (Table 1). Change in body weight was identical between moxonidine, hydralazine and untreated control groups, but the α-methyldopa group lost a slight amount of weight despite the lack of change in food intake.

Figure 1 shows systolic blood pressure as determined by tail cuff in treated and untreated control SHROB. Each of the agents reduced blood pressure nearly to normotensive levels, defined as systolic pressure < 150 mmHg. Note that all three antihypertensive agents were equally effective at reduction blood pressure in the SHROB model ($p < 0.001$, one-way ANOVA).

Metabolic parameters in the fasted state

Table 1 presents the metabolic characteristics in treated SHROB and untreated SHROB. The SHROB exhibits the plasma markers of metabolic syndrome X, including hyperinsulinemia, hyperglucagonemia and Type IV hyperlipidemia. Treatment with 4.0 mg/kg/d moxonidine significantly reduced fasting plasma insulin, glucagon, triglycerides, total cholesterol and FFA compared to untreated SHROB. α-Methyldopa trended to reduce fasting insulin, but this effect was not significant ($p > 0.05$). α-Methyldopa did not reproduce any of the metabolic effects seen after moxonidine treatment. Hydralazine significantly reduced fasting plasma glucagon and FFA compared to untreated SHROB, but had no other effect on fasting metabolic parameters.

Response to a glucose load

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Figure 2A shows plasma glucose in treated and untreated SHROB at various times after the glucose load. Consistent with previous results, SHROB showed glucose intolerance with fasting normoglycemia. Moxonidine significantly reduced the glucose response compared to untreated SHROB. However, α-methyldopa and hydralazine further impaired the glucose response compared to untreated SHROB ($p<0.05$). Figure 2B shows the glucose AUC in treated and untreated SHROB. Moxonidine significantly reduced the glucose AUC compared to untreated SHROB (13.6 ± 2.4 vs. 42.5 ± 9.9 g*min/dL). α-Methyldopa and hydralazine increased the glucose AUC compared to untreated SHROB (68 ± 26 and 110 ± 21 g*min/dL, respectively; $p<0.001$, one-way ANOVA).

Figure 3A illustrates the plasma insulin response in treated and untreated SHROB during an OGTT. The observed levels of insulin are 20-fold elevated compared to lean SHR controls (Velliquette et al., 2002). Moxonidine significantly facilitated the initial glucose induced insulin response at the first two time points compared to untreated SHROB ($p<0.001$). In contrast, α-methyldopa and hydralazine significantly blunted the insulin response during the first 120 min. Interestingly, the group treated with hydralazine showed a significant fall in plasma insulin during the first 240 min following a glucose load ($p<0.001$). Figure 3B represents the plasma insulin AUC in treated and untreated SHROB. Moxonidine significantly increased the insulin AUC compared to untreated SHROB ($p<0.01$). This was primarily an effect on the early insulin response during the first 60min (700 ± 160 vs. 13 ± 128 ng*min/mL, $p<0.001$). In contrast, chronic hydralazine significantly reduced the total plasma insulin AUC compared to untreated SHROB (-2.3 ± 1.0 vs. 3.9 ± 1.8 µg*min/mL) ($p<0.001$).

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Plasma C-peptide was measured as an index of insulin secretion, since plasma insulin reflects both the secretion and turnover of insulin. Figure 4A shows the plasma C-peptide response to the glucose load. Plasma C-peptide generally mirrored the insulin response in all groups. However, moxonidine lowered the fasting plasma insulin to C-peptide ratio suggesting that insulin turnover was greater after moxonidine treatment compared to untreated controls (see Table 1). Figure 4B illustrates the plasma C-peptide AUC for all groups. Chronic moxonidine treatment significantly increased the C-peptide AUC compared to untreated controls (1.25 ± .29 vs. 0.43 ± 0.14 μM*min) ($p<0.01$). As seen with insulin, hydralazine treatment inverted the normal response of C-peptide to a glucose load, resulting in a significant negative C-peptide AUC (-0.57 ± 0.23 μM*min) ($p<0.001$). These results suggest, in part, that moxonidine improves glucose tolerance by increasing the glucose induced insulin secretion, whereas α-methyldopa and hydralazine worsen glucose tolerance by inhibiting the insulin secretory response to a glucose challenge.

Figure 5A shows the plasma glucagon response to the glucose load in treated and untreated SHROB. As previously reported, glucagon shows a paradoxical rise in response to a glucose load in SHROB (Velliquette et al., 2002). Moxonidine significantly reduced this abnormal plasma glucagon response at all time points post load compared to untreated SHROB ($p<0.001$). In marked contrast, chronic treatment with α-methyldopa significantly potentiated the glucagon response to an oral glucose load, increasing glucagon by 2-fold at 30 min and nearly 3.5-fold at 60 min post load ($p<0.001$). Hydralazine also increased the glucagon response but only at 60min compared to untreated controls ($p <0.05$). Plasma glucagon AUC is presented in Figure

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5B. Moxonidine significantly reduced the plasma glucagon AUC compared to untreated controls (5.0 ± 0.6 vs. 10 ± 1.3 ng*min/mL; $p<0.05$; one-way ANOVA), while both α-methyldopa and hydralazine elevated the glucagon AUC ($p<0.01$). This finding suggests that the mixed I_1R/α_2AR agonist, moxonidine, influences both glucose induced insulin and glucagon secretion but in opposite directions. These effects were not duplicated by the specific α_2AR agonist α-methyldopa and in fact this agent had opposite effects.

Figure 6 shows the percent change in FFA levels after an oral glucose load in treated and untreated SHROB. As previously observed, control SHROB fail to show the expected suppression of FFA following a glucose load (Velliquette et al., 2002). Moxonidine significantly reduced FFA levels at all time points measured post glucose load ($p<0.05$). The α-methyldopa treated group did not differ from untreated SHROB. Hydralazine significantly elevated FFA levels 60 min post glucose load by 41% ($p<0.05$). These results suggest that moxonidine, but not α-methyldopa or hydralazine, decreased FFA, possibly reflecting improved adipose tissue insulin sensitivity. These data reinforce the hypothesis that stimulation of I_1R and not sympathoinhibition, increases in blood flow or activation of α_2AR mediate changes in lipid metabolism following antihypertensive treatment with imidazoline agents such as moxonidine.

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Discussion

We showed that three antihypertensive agents in clinical use had contrasting effects on glucose tolerance in an insulin resistant rodent model, despite equal effectiveness in lowering blood pressure. The mixed I_1R/α_2AR agonist, moxonidine, improved plasma markers of metabolic syndrome X and improved all measured parameters of glucose tolerance. The α_2AR agonist, α-methyldopa, did not significantly improvement plasma markers of metabolic syndrome X and actually worsened glucose tolerance compared to untreated controls. Hydralazine, a direct vasodilator, significantly reduced fasting plasma glucagon and FFA, yet significantly exacerbated all measured parameters of glucose tolerance.

Notably, two agents from the same class, centrally acting sympatholytic agents, had opposite effects on glucose, insulin and glucagon levels. These contrasting effects are not consistent with a hemodynamic model to account for enhanced glucose metabolism following antihypertensive therapy, as all three lower blood pressure by reducing peripheral resistance. Increased blood flow to metabolic tissues has been hypothesized to improve glucose homeostasis during antihypertensive treatment by improving substrate and hormones delivery (Julius et al., 1992). This hypothesis suggests that any agent that increases blood flow to metabolic tissues would improve glucose homeostasis. The present data are more consistent with the importance of specific receptor-mediated effects associated with individual agents. Thus, the improved glucose tolerance induced by moxonidine may be mediated by specific receptors.

Based on current theories regarding the metabolic actions of antihypertensive agents, we identified four possible hypotheses each predicting a different outcome in

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our tests of three different agents. The hemodynamic hypothesis predicted that each of the three agents would improve glucose disposal equally by lowering peripheral resistance and improving glucose and insulin delivery to active tissues. Because only one of the agents was effective and the other two actually worsened glucose tolerance, the hemodynamic hypothesis cannot account for the results. The sympathoadrenal hypothesis, which postulates a primary role for the sympathoadrenal system in the control of glucose metabolism, predicts that the two sympathoinhibitory agents, moxonidine and α-methyldopa, would improve glucose tolerance. Only moxonidine was effective, whereas α-methyldopa actually worsened glucose tolerance, which is not consistent with an overriding importance of sympathoadrenal inhibition. Nonetheless, the sympathoadrenal hypothesis correctly predicts the deleterious effect of hydralazine on glucose disposal, because this agent is known to evoke reflex sympathoadrenal activation. Thus, the present data are consistent with contributory role of sympathoadrenal activity in the effects of some antihypertensive agents on glucose tolerance. The mechanisms for improvements in glucose metabolism following inhibition of sympathetic tone have been recently reviewed. Reduced elevated sympathetic outflow might improve glucose tolerance by diminishing the actions of catecholamine to decrease insulin and increase glucagon secretion, stimulate glycogenolysis and gluconeogenesis, elevate FFA, and decrease skeletal muscle blood flow (Ernsberger et al., 1998).

The hypothesis most consistent with the data is the imidazoline receptor hypothesis, which proposes that direct activation of imidazoline receptors by moxonidine mediates the actions of this agent on glucose metabolism. A limitation of

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the present study is that we did not demonstrate blockade of moxonidine's effects with antagonists. Preliminary studies using osmotic minipumps to deliver drugs continuously indicated that doses of efaroxan high enough to antagonize the actions of moxonidine had effects when given alone (data not shown), consistent with previous reports of improved glucose metabolism and insulin secretion following efaroxan treatment (Berridge et al., 1992). Several mechanisms for a direct influence of I_1R on glucose metabolism are possible. Radioligand binding studies have identified I_1R in the endocrine pancreas and liver (Ernsberger et al., 1995), and liver localization has been confirmed by Northern blot analysis of the I_1R candidate gene (Piletz et al., 1999). An action of I_1R in the endocrine pancreas is the most likely explanation for the restoration of the early phase of insulin secretion induced by moxonidine treatment. Chronic treatment with moxonidine also increases expression and tyrosine phosphorylation of insulin receptor and insulin receptor substrate-1 in the liver, possibly through direct action (Ernsberger et al., 1999). We have recent found that an acute improvement in glucose tolerance can be induced by moxonidine if α_2AR are blocked (Velliquette et al., 2003). This improvement in glucose tolerance induced by a single dose of moxonidine could be blocked by efaroxan, implicating an I_1R mechanism. Similarly, the acute lipid lowering actions of moxonidine are blocked by efaroxan and are not affected by α_2AR blockade (Velliquette et al., 2003). Thus, based on prior results, the improvements in glucose and lipid metabolism observed in the present study after chronic treatment are mostly likely mediated by I_1R.

Some authors have proposed that all of the therapeutic actions of moxonidine and other imidazolines can be entirely accounted for by their activity at α_2AR (Szabo et

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al., 2001;Zhu et al., 1999). This α_2AR hypothesis is based on studies of centrally mediated cardiovascular responses, which can be mediated by either I_1R or α_2AR acting in synergy. In the present study, the α_2AR hypothesis predicts that moxonidine should have effects identical to the specific α_2AR agonist α-methyldopa. Notably, moxonidine and the active metabolite of α-methyldopa, alpha-methylnorepinephrine, have similar K_i values for the α_2AR (Ernsberger, 2000). Although α-methyldopa reduced blood pressure similarly to moxonidine, the metabolic outcomes were in marked contrast, with no decreases in circulating lipids and a worsening of glucose tolerance. Deterioration of glucose tolerance appeared to be the result of an attenuated early phase of insulin release in response to a glucose load and a large increase in glucagon secretion. The most likely mechanism is activation of α_2AR on pancreatic islets, which are known to inhibit insulin and promote glucagon secretion (Hirose et al., 1992). Chronic treatment with α-methyldopa has been reported to have negative affects on glucose homeostasis in humans (Benfield and Hunter, 1982) but has not been previously tested in rats. Moxonidine, even though it is a full agonist at α_2AR, did not elicit these responses and in fact elicted opposite effects. The simplest explanation for the contrasting effects of α-methyldopa and moxonidine is that a second non-adrenergic receptor is involved in the actions of moxonidine. Thus, the α_2AR hypothesis must be rejected as an explanation for the metabolic actions of imidazolines.

Consistent with previous studies from our laboratory using higher doses of moxonidine given for a longer period of time (8 mg/kg/d for 90d) (Ernsberger et al., 1999;Ernsberger et al., 1996), we noted improvements in multiple markers of metabolic syndrome X, including improved glucose tolerance, reduced fasting levels of insulin,

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triglycerides, cholesterol and FFA. However, our prior studies also found a decrease in food intake and decreased rate of weight gain. These effects on feeding and weight did not occur at the dose of 4 mg/kg/d in the present study. A limitation of our past studies was a possible independent effect of reduced food intake and body weight gain on metabolic parameters. We now show that the metabolic effects observed at the higher dose can be reproduced at 4 mg/kg/d independent of any alteration in caloric intake or body weight. The other two agents had no major impact on food intake or body weight. The small decrease in body weight induced by α-methyldopa failed to translate into significant improvements in glucose or lipid metabolism.

Possible etiologic factors underlying metabolic syndrome X have recently been identified. Cytokines such as TNF-α and IL-6 can be produced by adipocytes and may contribute to insulin resistance and metabolic syndrome X, at least in part through generation of reactive oxygen species (Fernandez-Real and Ricart, 2003). The relationship between I_1R and cytokines is unknown. However, its gene candidate shares several motifs with cytokine receptors (Piletz et al., 2000) and its signaling pathway overlaps those of cytokine receptors (Ernsberger, 2000). It is not known whether I_1R mediate pro- or anti-inflammatory actions. FFA mediate inflammatory actions on the vascular endothelium which may contribute to insulin resistance (Toborek et al., 2002). By chronically lowering FFA, moxonidine may make a small contribution to reducing inflammation through this pathway. It might be argued that because the SHROB rat model is a natural occurring leptin receptor knockout, the absence of leptin signaling might have blunted or augmented the actions of imidazolines. However, similar effects

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of moxonidine are seen in the fructose fed and lean SHR models (Ernsberger et al., 1996;Rosen et al., 1997), which have intact leptin receptors.

A recent study found improved insulin sensitivity during acute hydralazine infusion in anesthetized SHR rats, which was correlated with increased skeletal muscle blood flow (Pitre et al., 1999). The results were interpreted as supporting the hemodynamic hypothesis, which attributes the insulin resistance associated with hypertension to impaired delivery of blood to metabolically active tissues. In the present study, we found that chronic hydralazine treatment of obese animals form the same SHR genetic background not only failed to increase insulin sensitivity, but actually decreased it. The apparently contrasting effects of acute and chronic hydralazine may reflect the contribution of reflex sympathetic activation, which might be blunted in acute surgical preparations. Reflex stimulation of catecholamine release has been reported after hydralazine treatment in rats (Sanbar and de Romero, 1969). In humans, reflex sympathetic activation acutely induces insulin resistance (Jamerson et al., 1993)

In agreement with many clinical studies, blood pressure lowering agents do not necessarily affect comorbidities associated with hypertension such as glucose intolerance and hyperlipidemia. In fact, therapy for hypertension with a direct vasodilator actually worsens glucose metabolism in the SHROB model of metabolic syndrome X. Furthermore, reduced sympathetic outflow does not seem necessary for the therapeutic outcomes since α-methyldopa did not reproduce any of the benefits observed after moxonidine treatment. The results of this study suggest but do not establish that the I_1R component of moxonidine is responsible for the therapeutic benefits after chronic moxonidine treatment. The ability of moxonidine to activate α_2AR may limit its

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effectiveness in the treatment of glucose intolerance and insulin resistance. Development of more specific agonists at I_1R may lead to improved therapeutic agents for the treatment of metabolic Syndrome X.

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Acknowledgements

We thank Robin Mooney for her dedicated technical assistance.

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FOOTNOTES

*Supported by HL44514 from the NIH and by a grant from Solvay Pharmaceuticals, Hannover, Germany

[1]Submitted in partial fulfillment of the requirements for a doctorate in Nutrition from Case Western Reserve University School of Medicine.

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Figure Legends

Figure 1. Systolic blood pressure as determined by tail cuff in treated and untreated SHROB. For this and all subsequent figures, N = 14 for control SHROB, N = 15 for moxonidine treated, N = 12 for α-methyldopa treated and N = 10 for hydralazine treated. All three antihypertensive agents were equally effective at reduction blood pressure in the SHROB model. ***p<0.001 versus untreated control; one-way ANOVA.

Figure 2. Panel A shows **t**he plasma glucose response to a 6g/kg oral glucose load in 18h fasted treated and untreated SHROB. Untreated SHROB show mild glucose intolerance. Moxonidine significantly reduced the glucose response compared to untreated SHROB. However, α-methyldopa and hydralazine elevated the glucose response compared to untreated SHROB. *p <0.05; **p <0.01; ***p <0.001, two-way ANOVA. Panel B shows the glucose AUC in treated and untreated SHROB after the oral load. Moxonidine significantly reduced the glucose AUC compared to untreated SHROB, while α-methyldopa and hydralazine significantly elevated the AUC compared to untreated SHROB. ***p <0.001, one-way ANOVA.

Figure 3. Panel A shows the plasma insulin response to a 6g/kg oral glucose load in 18h fasted treated and untreated SHROB. Untreated SHORB show elevated fasting levels of insulin and a delayed exaggerated response to a glucose challenge. Moxonidine significantly improved the initial insulin response compared to untreated SHROB. α-methyldopa and hydralazine significantly blunted the insulin response during the first 120 min. In addition, hydralazine actually reduced insulin levels during the first 240 min. *p <0.05; **p <0.01; ***p <0.001, two-way ANOVA. Panel B shows the insulin AUC in treated and untreated SHROB after a glucose load. Moxonidine treatment

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significantly increased the insulin AUC compared to untreated SHROB. This was mainly due to an improved early phase of insulin secretion, in the first 120 min. Treatment of SHROB with α-methyldopa did not significantly alter the response to a glucose load, although it tended to decrease. Treatment with hydralazine resulted in a significant negative or inverted insulin AUC. ***** p <0.05; ****** p<0.001, one-way ANOVA.

Figure 4. Panel A shows the a C-peptide response to a 6g/kg oral glucose load in 18h fasted treated and untreated SHROB. Plasma C-peptide generally paralleled the insulin response. Animals treated with moxonidine showed robust glucose induced insulin secretion compared to untreated controls. In contrast, the α-methyldopa treated group showed a blunted insulin response, and hydralazine treatment inverted the insulin secretory response to glucose resulting in a fall in C-peptide after the load. ***** p <0.05; ****** p <0.01, two-way ANOVA. Panel B shows the C-peptide AUC in treated and untreated SHROB in response to a glucose load Moxonidine significantly increased the C-peptide AUC compared to untreated SHROB. Chronic hydralazine treatment resulted in a significantly negative C-peptide AUC. ****** p <0.01; ******* p<0.001, one-way ANOVA.

Figure 5. Panel A shows the plasma glucagon response to a 6g/kg oral glucose load in 18h fasted in treated and untreated SHROB. Untreated SHROB show a paradoxical increase in glucagon in response to a glucose load. Moxonidine significantly reduced the glucagon response at all time points compared to untreated SHROB. Treatment with α-methyldopa potentiated the glucagon response to a glucose load by 2-fold at 30 min and nearly 3.5-fold at 60 min post load. Hydralazine treatment elevated the glucagon response at 60min only. ***** p <0.05; ******* p <0.001, two-way ANOVA. Panel B shows the

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glucagon AUC in treated and untreated SHROB after a glucose load. Moxonidine significantly reduced the glucagon AUC compared to untreated SHROB. α-methyldopa and hydralazine significantly elevated the glucagon AUC compared to untreated SHROB. ***p<0.05; ****p<0.001, one-way ANOVA.

Figure 6. The percent change in plasma FFA levels after a 6g/kg oral glucose load in 18h fasted treated and untreated SHROB. Moxonidine significantly reduced plasma FFA levels at all time points. The α-methyldopa and hydralazine treated groups did not differ from untreated SHROB. ***p <0.001, two-way ANOVA.

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TABLE 1. Fasting plasma markers for metabolic syndrome X in treated and untreated SHROB.

	Untreated	**Moxonidine**	**α-methyldopa**	**Hydralazine**
	SHROB	SHROB	SHROB	SHROB
	(N=14)	(n=15)	(N=12)	(N=10)
Body weight change (g)	12.3 ± 4.1	9.4 ± 4.5	-15.0 ± 5.3*	11.2 ± 3.5
Average food intake (g/d)	27.1 ± 0.5	29.8 ± 0.8	26.8 ± 1.6	30.5 ± 1.1
Glucose (mg/dL)	112 ± 5.4	105 ± 4.1	111 ± 6.7	107 ± 3.4
Insulin (ng/mL	20 ± 3.7	11 ± 1.7*****	11 ± 2.6	17 ± 3.2
C-peptide (nM)	3.1 ± 0.07	3.1 ± 0.47	1.8 ± 0.37*****	3.3 ± 0.73
Glucagon (pg/mL)	114 ± 6.8	87 ± 11*****	114 ± 8.4	86 ± 4.7*****
Triglyceride (mg/dL)	466 ± 42	217 ± 17*****	368 ± 36	451 ± 93
Total Cholesterol (mg/dL)	379 ± 31	273 ± 39*****	354 ± 57	436 ± 67
FFA (mmol/L)	1.58 ± 0.11	1.29 ± 0.07*****	1.42 ± 0.11	1.16 ± 0.02*****

Data are means ± SEM. ***** Significantly different from Untreated SHROB, $p < 0.05$

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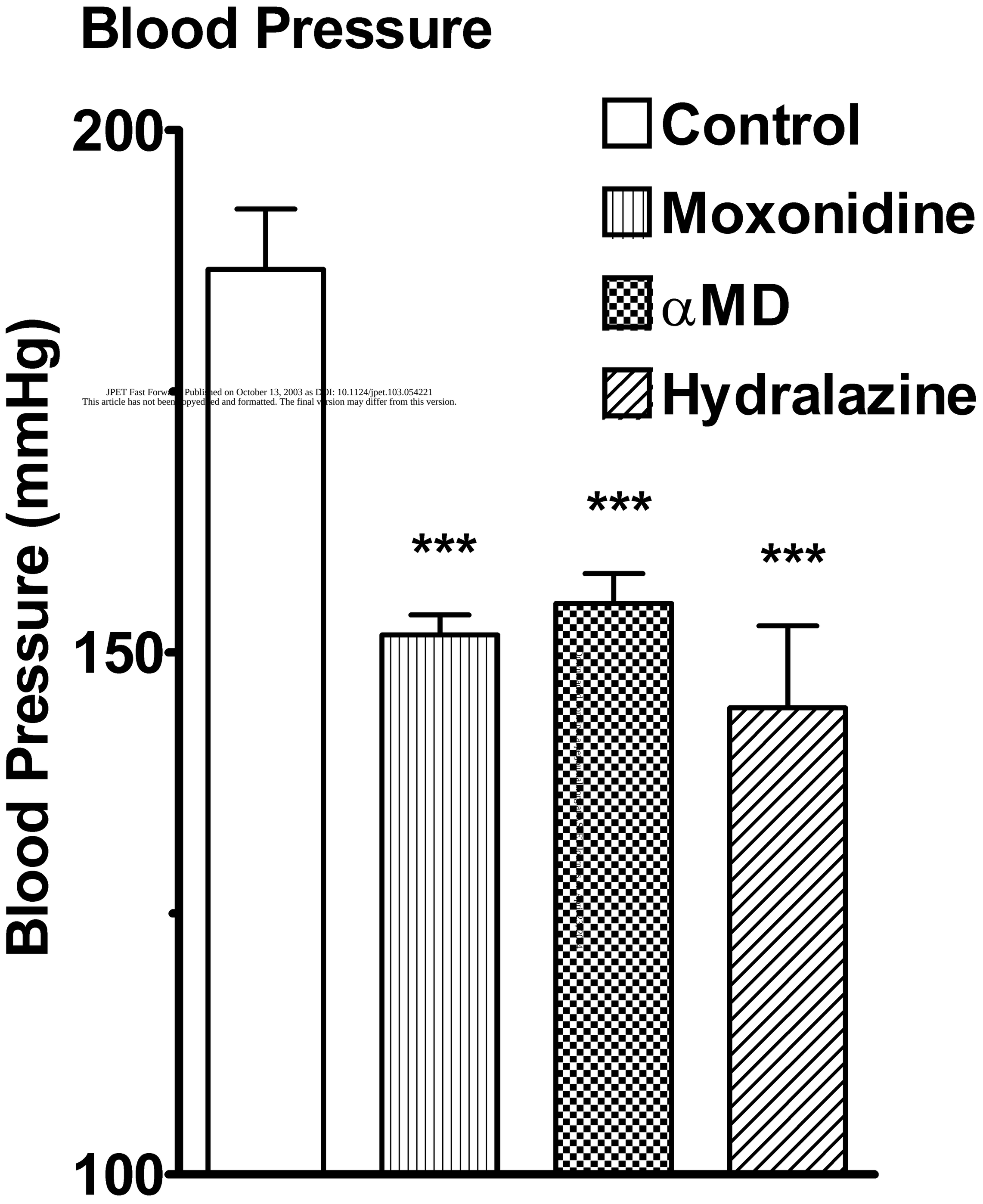

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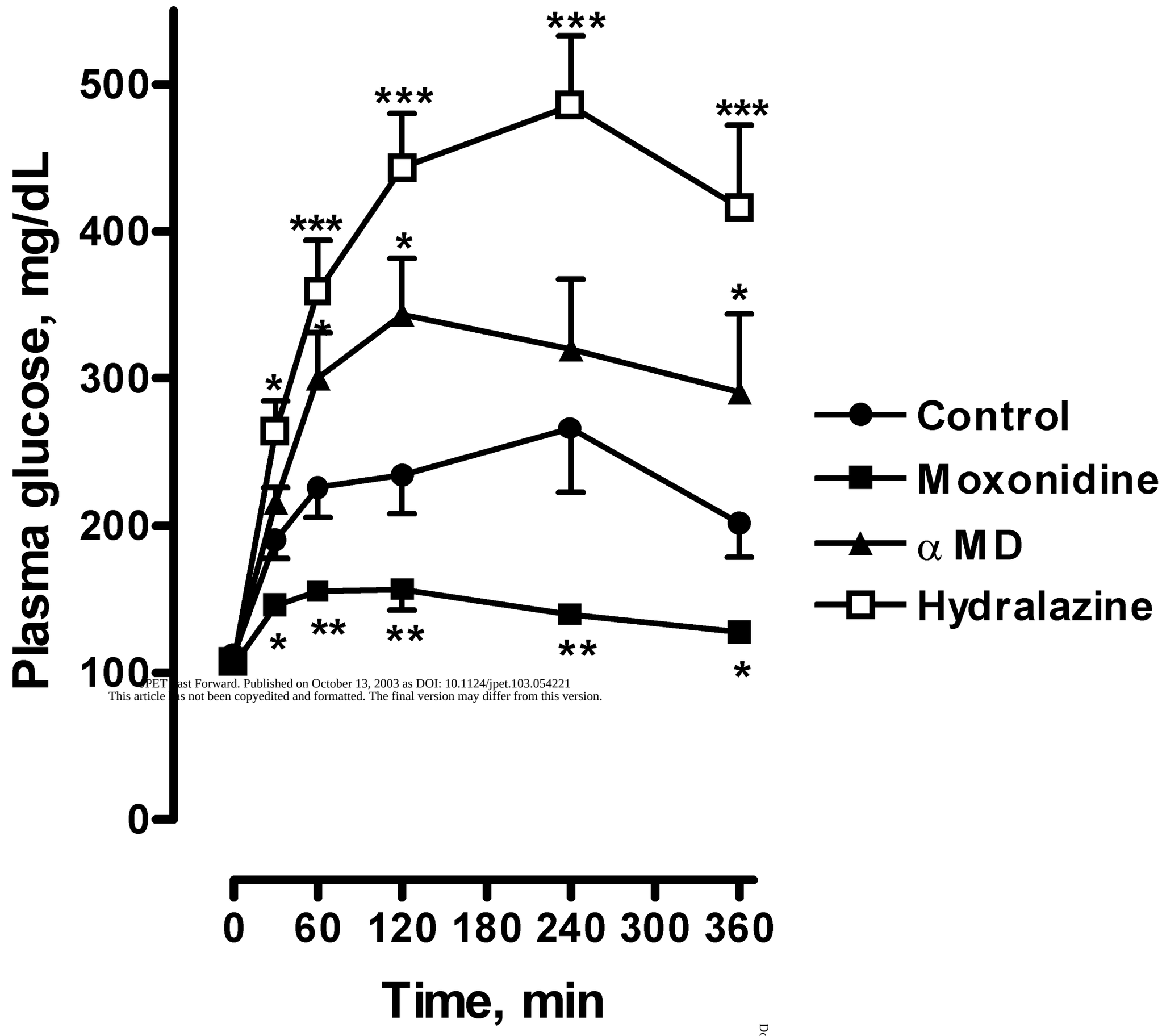

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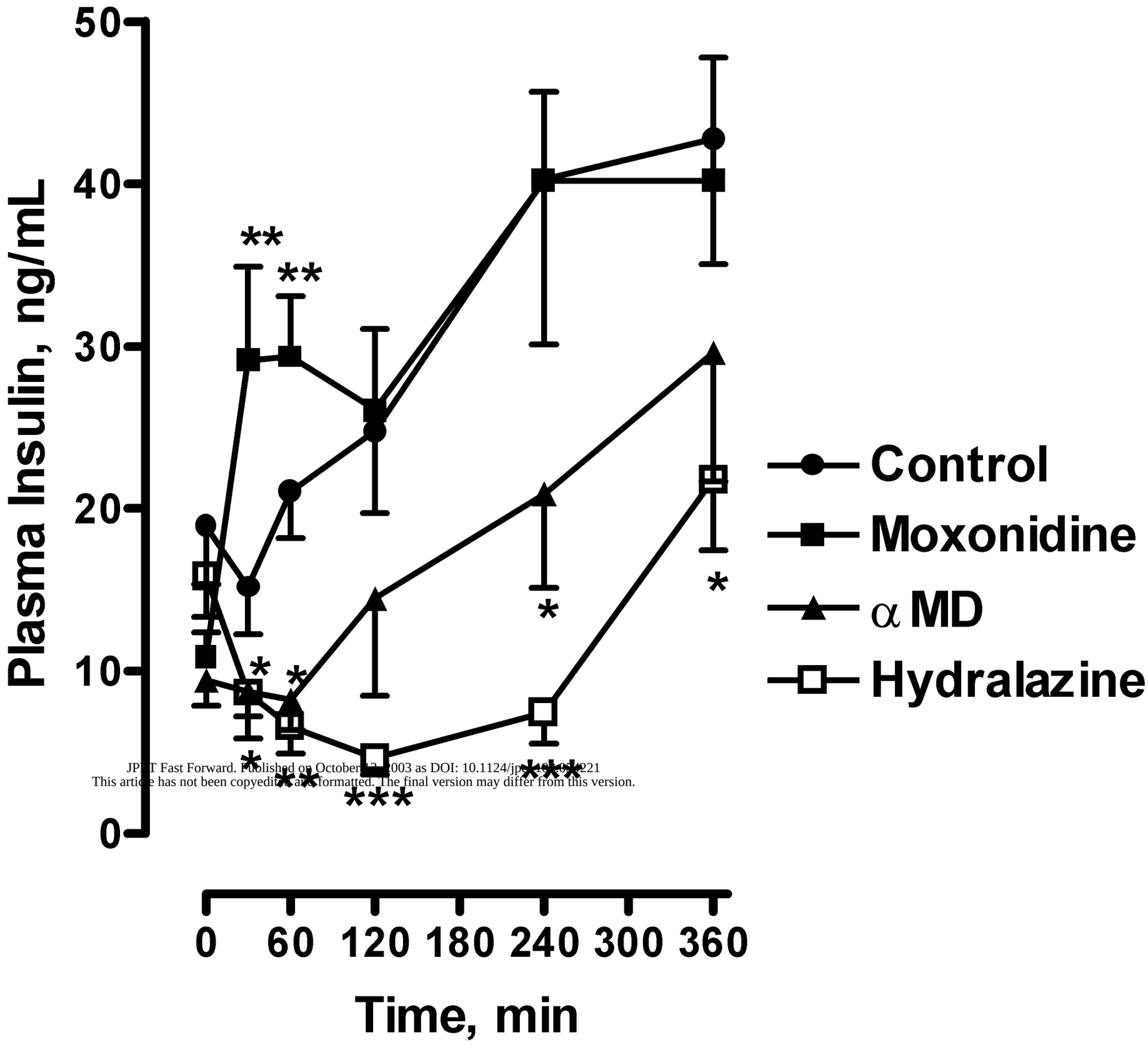
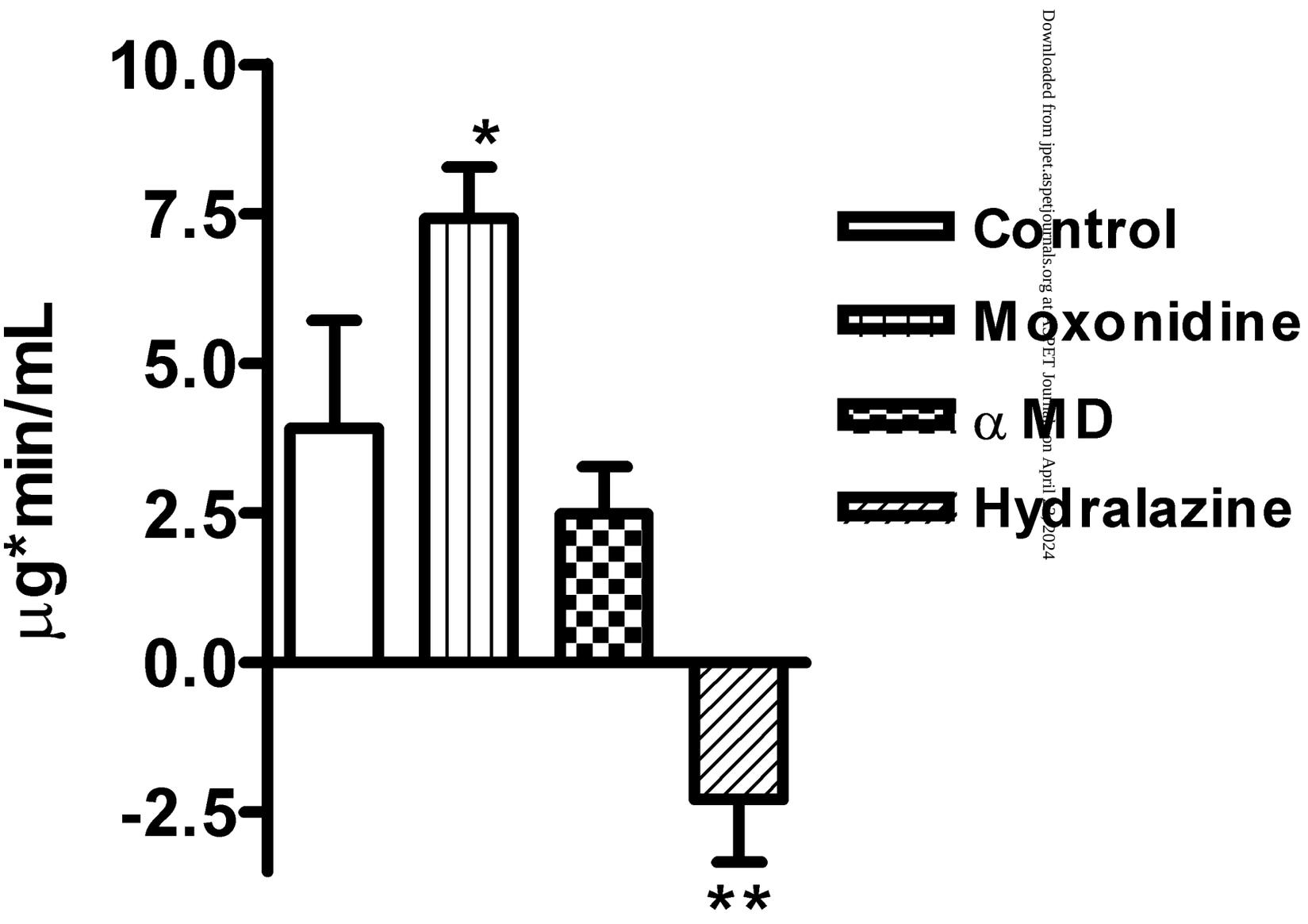
A. Plasma Insulin



B. Insulin AUC



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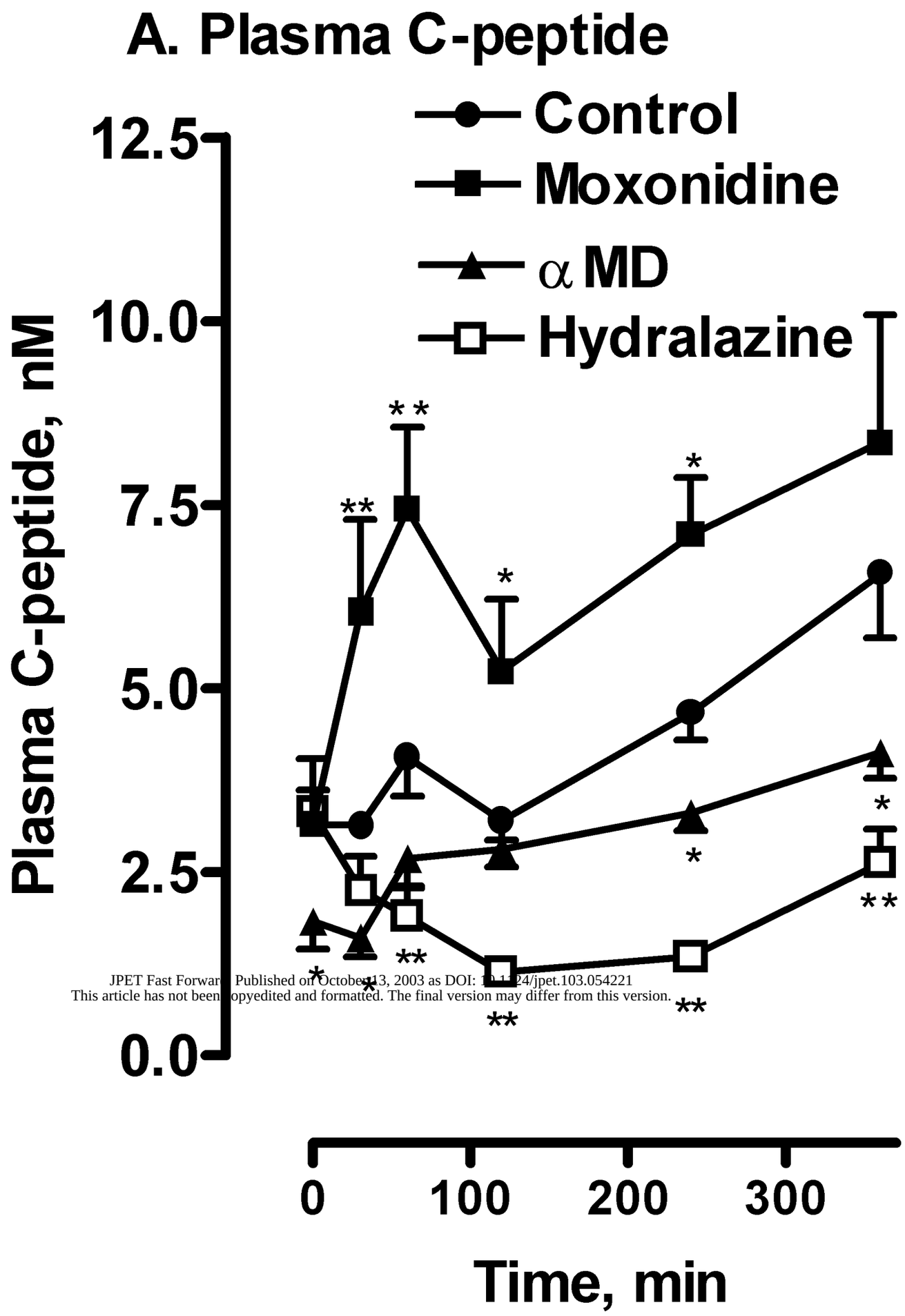

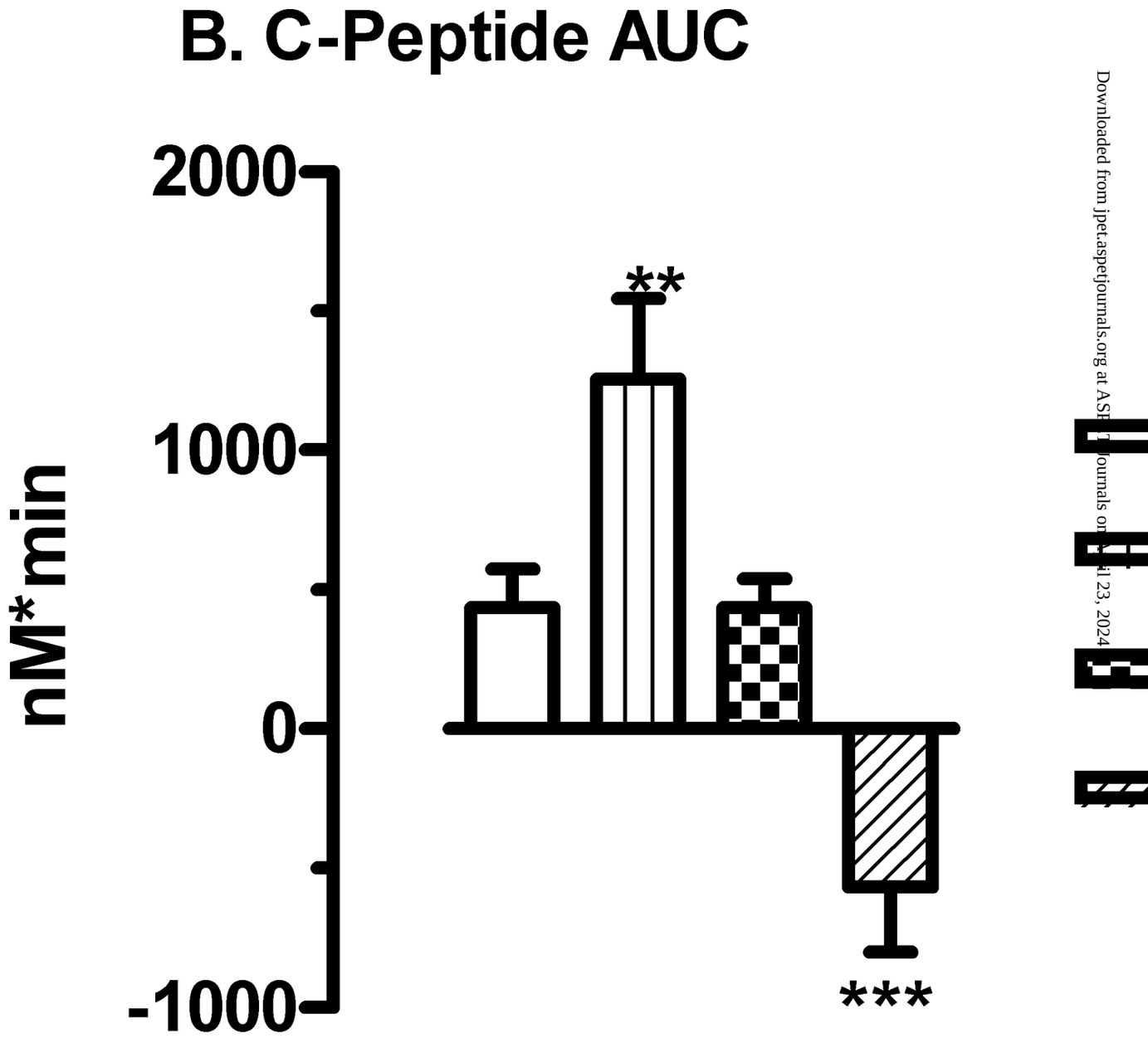

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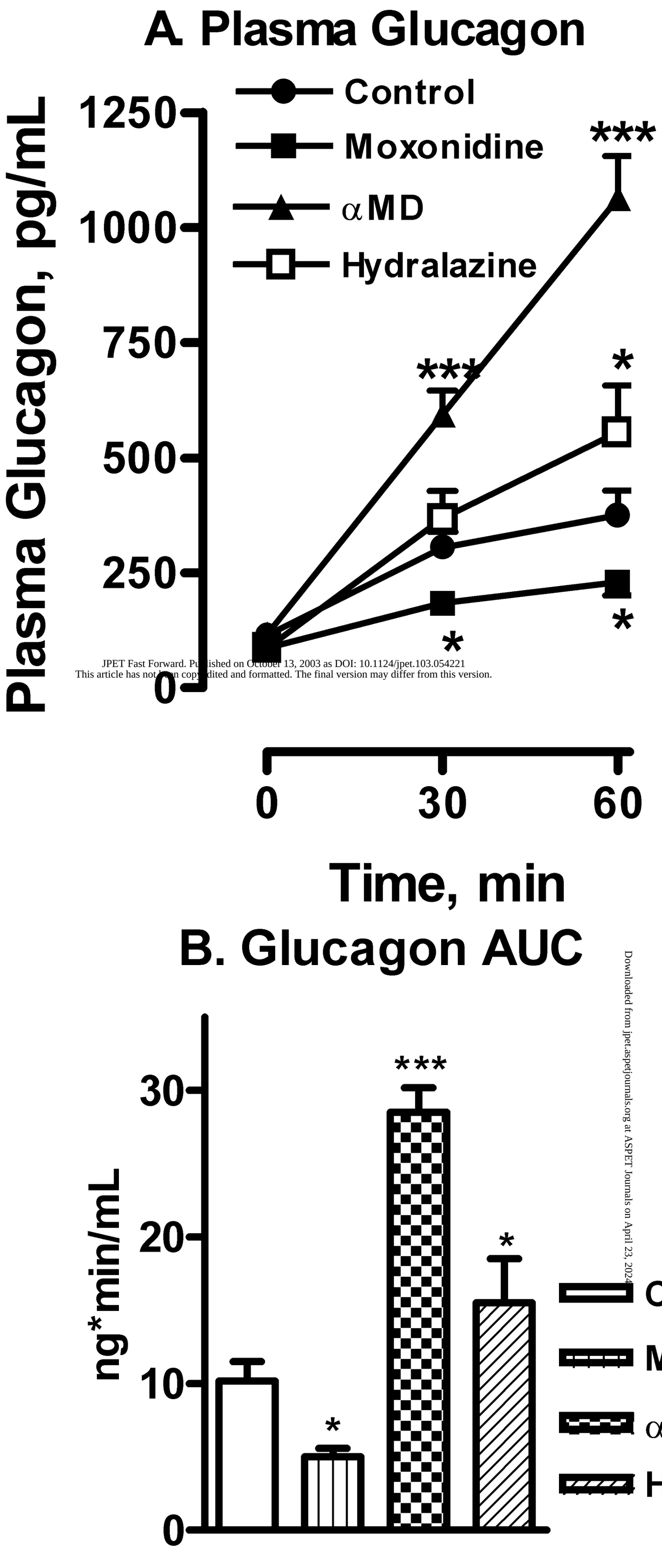

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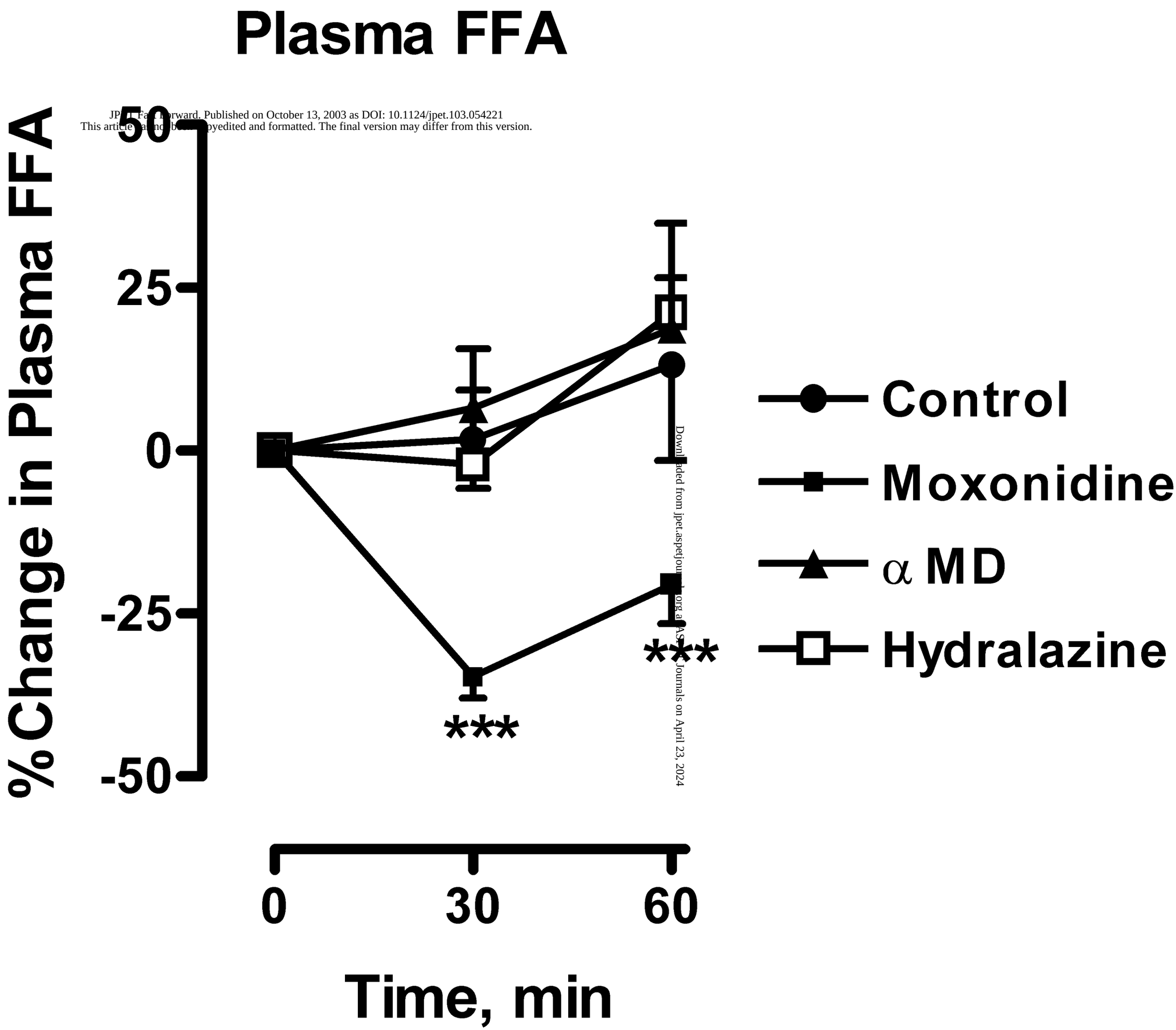

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