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Metabolic effects of a novel silicate inositol complex of the nitric oxide precursor arginine in the obese insulin-resistant JCR:LA-*cp* rat

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Abstract

Insulin resistance is a major contributor to macro- and microvascular complications, particularly in the presence of the metabolic syndrome, and is also associated with polycystic ovary syndrome. Impaired nitric oxide metabolism and endothelial function are important components of the vascular disease. Increasing the bioavailability of arginine, the precursor of nitric oxide, thus potentially offers protection against end-stage disease. We have recently demonstrated that dietary supplementation with a novel silicate inositol arginine complex reduces vasculopathy and glomerular sclerosis in the insulin-resistant JCR:LA-*cp* rat. The objective of this study was to address the absorption of, and the underlying metabolic alterations caused by, the arginine silicate inositol complex and arginine HCl (as a reference agent) in obese insulin-resistant male and female JCR:LA-*cp* rats. Male and female rats were treated with the preparations at 1.0 mg/(kg d) (expressed as arginine HCl) from 8 to 12 and 12 to 18 weeks of age, respectively. Obese female, but not male, rats treated with the arginine silicate inositol complex showed a reduced rate of weight gain without concomitant reduction in food intake. Plasma silicon levels were raised very significantly in arginine silicate–treated rats, consistent with significant absorption of the complex. In male rats, arginine levels were elevated by treatment with arginine silicate only; and female rats responded to both preparations. Plasma concentrations of oxides of nitrogen in rats treated with the silicate complex showed a dimorphism, decreasing in male and increasing in female rats. Fasting insulin levels were elevated in male rats treated with the arginine silicate complex, whereas fasting and postprandial insulin levels were decreased in female rats. Furthermore, female, but not male, rats treated with either of the arginine preparations showed significant reductions in cholesterol, triglyceride, and phospholipid concentrations. We conclude that the arginine silicate inositol complex is absorbed efficiently, raising plasma arginine levels, and is more biologically effective than the free amino acid hydrochloride. This has different beneficial metabolic effects in both sexes of an animal model of insulin resistance and cardiovascular disease, consistent with reduction in end-stage disease.

1. Introduction

The metabolic syndrome is a major and increasingly common cause of cardiovascular morbidity and mortality worldwide. A crucial feature of this disorder is the development of insulin resistance and consequent hyperinsulinemia [1,2]. The hyperinsulinemia seems to be a major determinant of the vasculopathy, atherosclerosis, and ischemic cardiovascular disease that are strongly associated with the metabolic syndrome [3,4]. In addition, there is increasing evidence that insulin resistance is etiologically associated with other macro- and microvascular-related diseases including polycystic ovary syndrome [5,6]. Clearly, reduction in insulin resistance, and therefore prevention of hyperinsulinemia, offers the prospect of important reduction in macro- and microvascular diseases and remains an attractive interventional approach. In addition to pharmacological approaches, effective treatments may include changes in diet, food intake, and physical activity. However, these have proven difficult to implement clinically, especially in situations where the metabolic, vascular-related syndromes are well established.

Vascular dysfunction and damage have been shown to be associated with impaired endothelial nitric oxide (NO) metabolism and function [4,7,8]. Nitric oxide is synthesized,

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in various cells, through the action of nitric oxide synthase (NOS) on the amino acid arginine, with the endothelial isoform eNOS playing a critical role [7]. Increasing the availability of the NO precursor arginine thus offers potential enhancement of vascular function and protection against end-stage disease processes. To date, the clinical efficacy of dietary arginine supplementation, in both humans and animal models of cardiovascular disease, has been limited [9]. Although the reasons for this remain debatable, a contributing factor may be use of poorly absorbed formulations with consequent low bioavailability of arginine. Alternative explanations could involve antagonism of eNOS by asymmetric dimethyl arginine or depressed levels of the NOS cofactor tetrahydrobiopterin [10,11].

Consistent with this hypothesis, we have recently demonstrated that a novel arginine silicate inositol complex (arginine silicate) is able to normalize the hypercontractile response of the aorta and coronary arteries in a model of obesity and insulin resistance, the JCR:LA-*cp* rat [12]. This unique strain spontaneously develops the range of the dysfunction and pathophysiology associated with the metabolic syndrome in humans [13]. The novel arginine silicate complex improved aortic and coronary artery function and significantly reduced glomerulosclerosis associated with the obese insulin-resistant phenotype. We hypothesize that treatment with exogenous arginine, in an efficiently absorbed form, improves vascular function and reduces nephropathy and cardiovascular disease, via metabolic mechanism(s) independent of insulin concentration. The arginine silicate preparation has physicochemical properties that increase the rate of absorption of arginine across the intestinal tract, compared with arginine HCl, and has been shown to be nontoxic in several ex vivo test systems [14-16].

Recent studies have shown that the male *cp*/*cp* rat at 12 weeks of age, when insulin levels are very high, has increased urinary albumin excretion and significant glomerular sclerosis [12]. Whereas the male *cp*/*cp* rats have more or less normal testosterone levels and testicular function, the *cp*/*cp* female rats, even at an early age, have polycystic ovaries and are functionally sterile [13]. Obese female rats also have elevated levels of testosterone and reduced levels of estrogen [17]. The female rats have lower plasma insulin levels, higher triglyceride levels, milder vascular disease, and lower incidence of ischemic myocardial lesions [18,19]. As in humans, these differences between male and female rats dictate that the sexes be used separately to look at different aspects of the metabolic syndrome.

We have investigated the metabolic effects of the arginine silicate inositol complex that has been shown to be biologically effective against micro- and macrovascular disease in the *cp*/*cp* rat. The present study assessed the efficiency of absorption and the efficacy of the arginine silicate inositol complex in improving the metabolic status of obese insulin-resistant male and female JCR:LA-*cp* rats using arginine HCl as a reference preparation in addition to nontreated control animals.

2. Methods

2.1. Animals and treatment

Male and female rats of the JCR:LA-*cp* strain were raised and housed in our established breeding colony at the University of Alberta, as described previously [20]. Rats homozygous for the autosomal recessive *cp* gene (*cp*/*cp*) are obese, whereas lean rats (+/?) are a 2:1 mix of heterozygotes (*cp*/+) and homozygote normals (+/+). Rats that are *cp*/*cp* spontaneously develop obesity, hyperlipidemia, profound insulin resistance, glomerular sclerosis, and atherosclerosis with enhanced vascular contractility and reduced vascular relaxation [21-25]. Animals that are heterozygous (*cp*/+) or homozygous normal (+/+) are indistinguishable, lean, and metabolically normal. The care of the animals and the experimental procedures were in conformity with the guidelines of the Canadian Council on Animal Care and subject to prior institutional review and approval.

At 7 weeks of age, the animals were conditioned to a sham tail-bleeding procedure and randomized to 1 of 2 treatment diets or control diet. Treatment diets were supplemented with either arginine HCl at a dose of 1 mg/(kg d) or arginine silicate inositol complex at a dose of 1.81 mg/(kg d) (giving a supplement of arginine of 4.75 mmol/[kg d]) and were provided to the male rats from 8 to 12 weeks of age. The female rats were subject to an identical regimen, but entered into the experimental protocol at 11 weeks of age and were treated from 12 to 18 weeks. This reflects an equivalent period, subsequent to onset of insulin resistance, to that of male *cp*/*cp* rats, which develop an earlier and more severe insulin resistance and hyperinsulinemia. Rats were weighed and their food consumption was measured twice a week during the intervention period. Arginine preparations were incorporated into powdered feed (Rodent Diet 5001; PMI Nutrition International, Brentwood, MO), and the concentration was adjusted weekly so as to maintain the desired dose of each agent on a gram per kilogram body weight basis [26]. Control groups, *cp*/*cp* and +/?, were given feed that was prepared using the same protocol with the exception of the incorporation of arginine.

2.2. Insulin and glucose metabolism

The metabolism of insulin and glucose in the *cp*/*cp* rat is abnormally responsive to stress or disturbance, and blood samples are routinely collected from conscious rats under a specific protocol during the dark (active) phase of their diurnal cycle to reduce variability. Meal tolerance tests were performed at 12 weeks of age in the male rats and 18 weeks of age in the female rats, following a standardized protocol [21], but with only 3 blood samples taken.

2.3. Materials

The arginine silicate inositol complex was supplied by Nutrition 21, Purchase, NY. This material, on assay,

contained 45.8% arginine, 31% inositol, and 8.6% silicon. Reagents and chemicals, including arginine hydrochloride (≥98% by thin-layer chromatography assay), were obtained from Sigma Chemical (Oakville, Ontario).

2.4. Analytical methods

Plasma glucose was measured by the use of a rapid glucose oxidase technique (Beckman Instruments, Brea, CA). Insulin was assayed by a double antibody radioimmunoassay technique (Kabi Pharmacia, Uppsala, Sweden) and rat insulin standards. Plasma arginine concentrations were measured using a Beckman 6300 Amino Acid Analyzer. Plasma total lipid profile was performed using the gas chromatographic technique of Kuksis et al [27]. Plasma silicon concentrations were determined using electrothermal atomic absorption spectrometry [28]. Nitrite/nitrate (NO_x) concentrations were determined using the method of Greene et al [29], which is based on the Griess reaction that reduces nitrate to nitrite using a copper/cadmium catalyst.

2.5. Statistical analysis

Results are expressed as mean ± SEM and were analyzed using SigmaStat (Jandel Scientific, San Rafael, CA) and plotted using SigmaPlot (SPSS, Chicago, IL). Data were compared using 1-way analysis of variance, followed by multiple comparison tests or, for body weight data, by 2-way analysis of variance. A value of $P < .05$ was taken as being statistically significant.

3. Results

3.1. Food intake and body weight

Fig. 1 shows the food intake and body weights of male rats from 8 to 12 weeks of age. The *cp*/*cp* male rats consumed 35 to 40 g of food per day compared with about 22 g per day for the +/? rats. There was no difference in food intake between any of the *cp*/*cp* groups, control, and those supplemented with arginine HCl or arginine silicate complex ($P > .05$). Body weights of +/? rats were significantly less than those of the *cp*/*cp* rats from the entry into the experimental protocol to the end. The body weight of the *cp*/*cp* rats, of all groups, was essentially identical; and there was no significant effect of either of the arginine preparations on body weight of the *cp*/*cp* male rats.

Food intake and body weights of the female rats are shown in Fig. 2. As observed in the *cp*/*cp* male rats, there was no significant difference in food intake of the control and arginine-treated *cp*/*cp* female rats. Similarly, although all groups of *cp*/*cp* rats had virtually identical initial body weights, arginine silicate–treated *cp*/*cp* female rats had a significantly lower rate of weight gain ($P < .001$). In contrast, female rats treated with arginine HCl did not differ in body weight from the *cp*/*cp* control group.

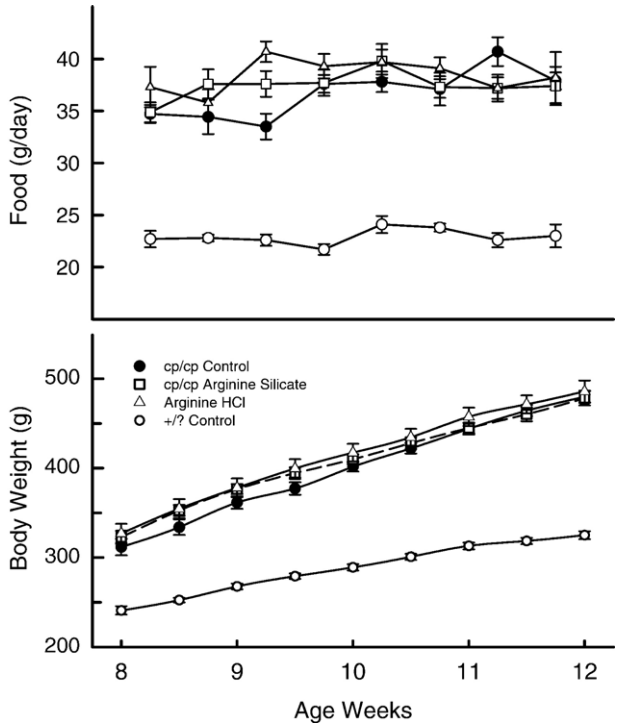

Fig. 1. Body weight and food intake of male JCR:LA-*cp* rats treated with arginine silicate inositol complex and arginine HCl. Data are mean ± SEM; 8 animals in each group. There were no significant differences in body weight or food intake between the *cp*/*cp* groups.

3.2. Plasma silicon concentrations

Plasma silicon concentrations (Fig. 3) were measured in the rats at the end of the treatment periods as an index of the efficacy of absorption of the arginine silicate inositol complex. The arginine silicate–treated rats of both sexes had markedly higher plasma silicon concentrations than the untreated controls or the arginine HCl–treated rats. The +/? rats (both male and female) had lower silicon concentrations than the *cp*/*cp* control animals ($P < .001$). The apparently lower silicon concentrations of the *cp*/*cp* female rats compared with the male rats were not significant ($P = .057$).

3.3. Plasma arginine concentrations

The plasma concentrations of free arginine are also shown in Fig. 3. Female *cp*/*cp* rats had lower arginine levels than the male *cp*/*cp* rats ($P < .05$). The concentrations of arginine were significantly higher in the rats treated with arginine silicate, in both male and female groups, compared with those in the *cp*/*cp* control rats ($P < .05$). There were smaller increases in the plasma arginine levels in rats treated with arginine HCl that were significant only in the female animals ($P < .01$).

3.4. Plasma NO_x concentrations

The plasma NO_x concentration provides an index of the total metabolic flux of NO and is shown in Fig. 4. The +/? female rats had lower NO_x concentrations than the +/? male

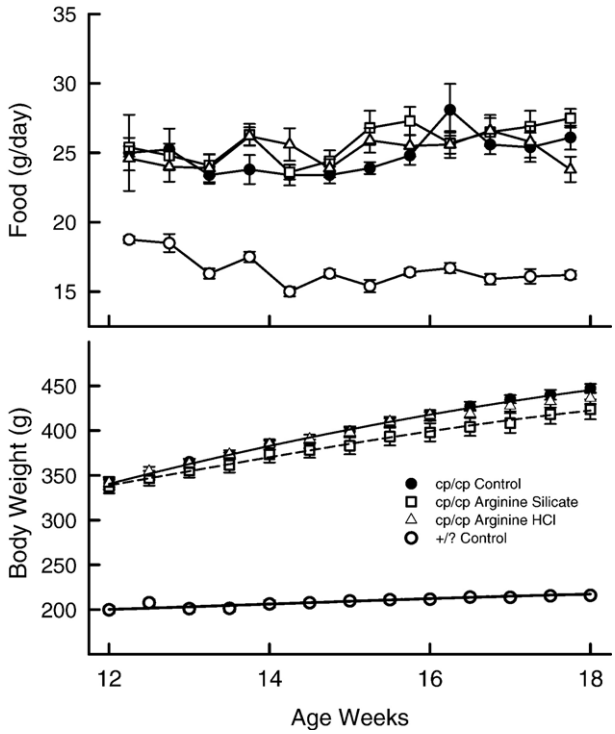

Fig. 2. Body weight and food intake of female JCR:LA-*cp* rats treated with arginine silicate inositol complex and arginine HCl. Data are mean ± SEM; 8 animals in each group. There were no significant differences in food intake between the *cp*/*cp* groups. The arginine silicate–treated *cp*/*cp* group showed a significantly lower rate of body weight gain than the *cp*/*cp* control group ($P < .001$).

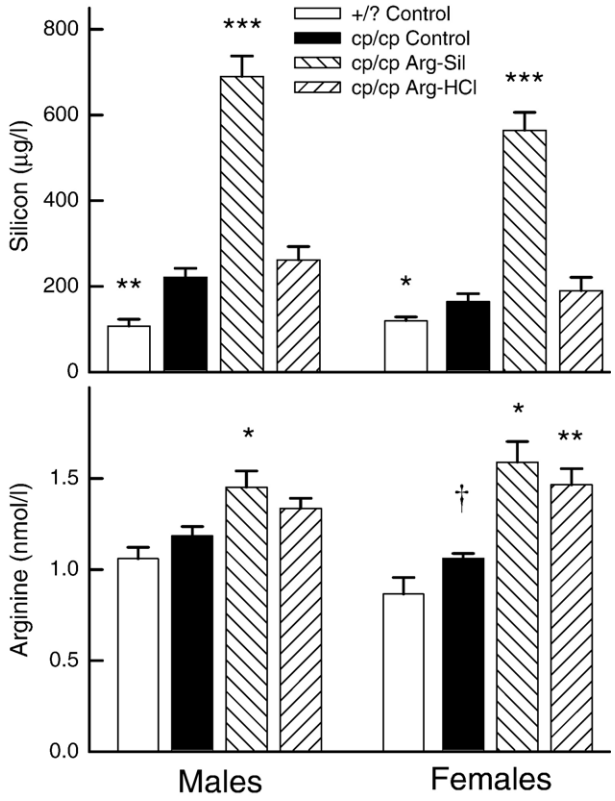

Fig. 3. Plasma silicon and free arginine concentrations in JCR:LA-*cp* rats treated with arginine silicate inositol complex and arginine HCl. Data are mean ± SEM; 8 animals in each group. *$P < .05$, **$P < .01$, ***$P < .001$ vs *cp*/*cp* control group; †$P < .05$ vs male *cp*/*cp* control group.

as well as the *cp*/*cp* female rats. The apparently lower NO_x levels of the *cp*/*cp* female rats compared with those of the *cp*/*cp* male rats were not significant ($P = .066$). Male *cp*/*cp* rats treated with arginine silicate showed significantly lower levels of NO_x than the *cp*/*cp* controls, whereas NO_x levels in arginine HCl–treated rats were not different. In contrast, female rats treated with arginine silicate had significantly higher NO_x levels; and again, arginine HCl–treated rats were not different from controls.

3.5. Plasma insulin and glucose concentrations

Fasting plasma insulin concentrations in male *cp*/*cp* rats (0 minute in the meal tolerance test) were markedly higher than those of the +/? controls (Fig. 5). Interestingly, in the fasting state, male *cp*/*cp* rats treated with the arginine silicate complex, but not arginine HCl, had elevated plasma insulin levels ($P < .05$). However, the postprandial insulin levels of the arginine-treated male rats (at both 30 and 60 minutes) were not significantly different from the *cp*/*cp* controls. Arginine HCl–treated male rats showed significantly higher glucose levels than both the *cp*/*cp* controls and arginine silicate–treated male rats at the 60-minute time point.

Female *cp*/*cp* rats characteristically have moderately elevated insulin concentrations (both fasting and postprandial) compared with +/? female rats, but significantly lower than those of the *cp*/*cp* male rats, reflecting a less severe state of insulin resistance (Fig. 6). Unexpectedly, treatment of *cp*/*cp* female rats with both arginine HCl and arginine silicate resulted in a significant reduction in fasting insulin levels (Fig. 6). In addition, at both 30 and 60 minutes after the meal tolerance test, female *cp*/*cp* rats treated with arginine silicate

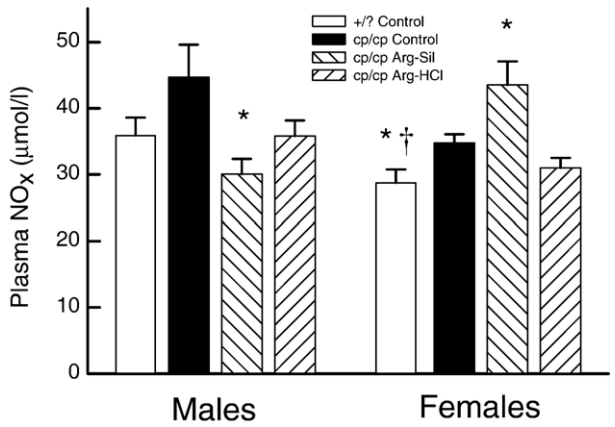

Fig. 4. Plasma NO_x concentrations in JCR:LA-*cp* rats treated with arginine silicate inositol complex and arginine HCl. Data are mean ± SEM; 8 animals in each group. *$P < .05$ vs *cp*/*cp* control group; †$P < .05$ vs *cp*/*cp* male group.

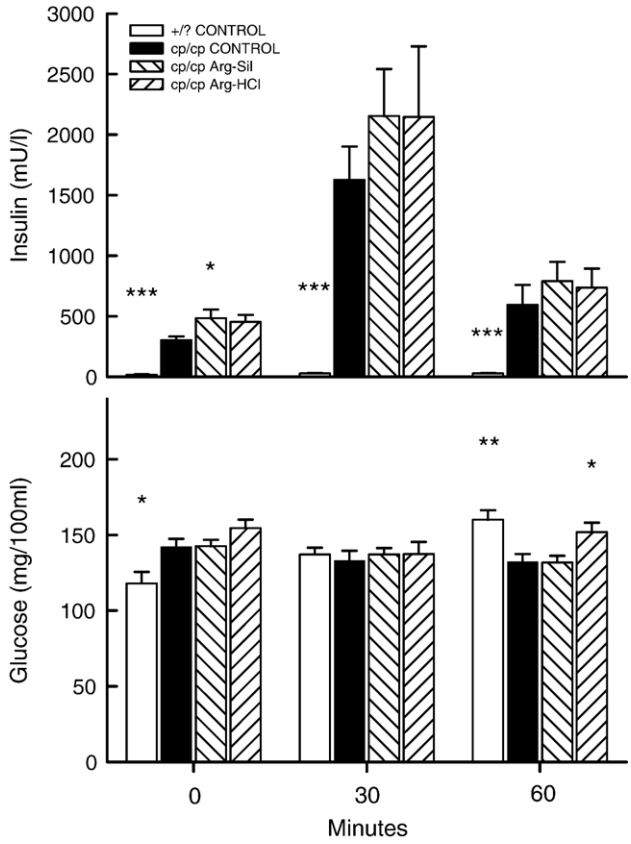

Fig. 5. Plasma insulin and glucose concentrations in 12-week-old male JCR:LA-*cp* rats during the meal tolerance test. Data are mean ± SEM; 8 animals in each group. * $P < .05$, ** $P < .01$, *** $P < .001$ vs *cp*/*cp* control group.

had substantially lower insulin levels ($P < .05$ and $P < .01$, respectively) compared with *cp*/*cp* control female rats. Consistent with this, the 30-minute postprandial glucose concentration in female rats treated with both arginine preparations was significantly reduced compared with that in *cp*/*cp* female controls, an effect that was no longer evident by 60 minutes.

3.6. Whole serum lipid concentrations

Table 1 shows the condensed results of the total lipid profile. Lipid concentrations were substantially lower overall in the +/? rats of both sexes (Table 1). Male *cp*/*cp* rats treated with arginine HCl showed a modest reduction in phospholipid and triglyceride concentrations ($P < .05$), an effect not seen in the arginine silicate–treated rats. The apparent reduction in cholesterol concentrations was not significant ($P = .064$ or greater). Female *cp*/*cp* rats had significantly elevated levels of unesterified cholesterol, phospholipids, and, particularly, triglycerides compared with the +/? controls, reflecting their greater very low-density lipoprotein (VLDL) hypertriglyceridemia. Female rats treated with either of the arginine preparations showed significantly lower levels of unesterified cholesterol, phospholipids, and triglycerides. Curiously, the female *cp*/*cp* rats treated with the arginine silicate complex were the only group to demonstrate an increase in cholesterol ester concentration ($P < .05$).

4. Discussion

The rationale of this study was that dietary supplementation with arginine could enhance the NO metabolism in individuals with the obesity/insulin resistance syndrome and thereby ameliorate the associated vascular dysfunction. Indeed, we have recently reported that treatment of *cp*/*cp* male rats with the arginine silicate inositol complex does significantly reduce the vascular dysfunction and severity of glomerular sclerosis [12]. We hypothesized that the arginine silicate inositol complex is more readily absorbed and therefore potentially more effective than the reference preparation, arginine HCl. The data are consistent with greater absorption and bioavailability of arginine from the silicate inositol complex, particularly in the female rats, and consistent with the hypothesis that the beneficial macro- and microvascular effects previously reported [12] are due to enhanced NO metabolism.

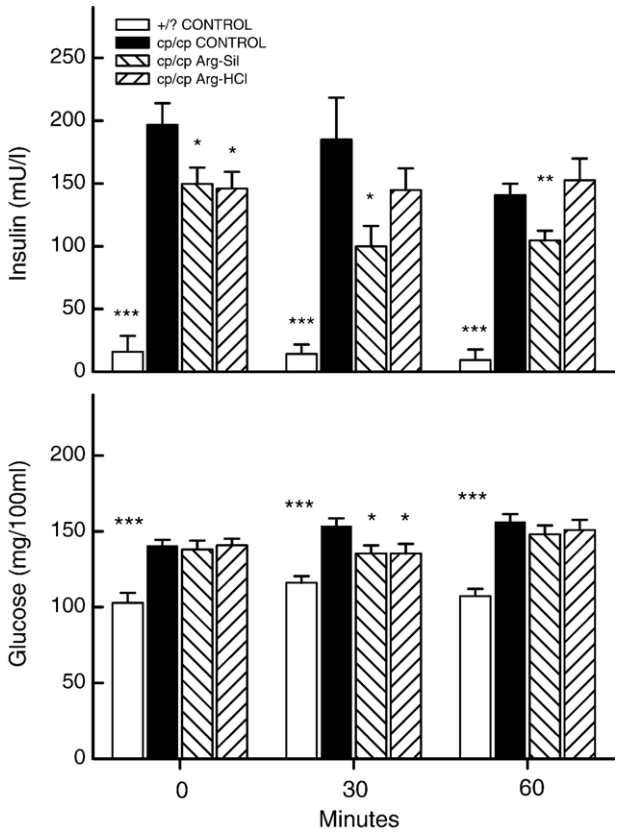

Fig. 6. Plasma insulin and glucose concentrations in 18-week-old female JCR:LA-*cp* rats during the meal tolerance test. Data are mean ± SEM; 8 animals in each group. * $P < .05$, ** $P < .01$, *** $P < .001$ vs *cp*/*cp* control group.

Table 1
Whole serum lipid concentrations in JCR:LA-*cp* rats treated with arginine

	Cholesterol	Cholesteryl esters	Total cholesterol	Phospholipids	Triglycerides
Male rats					
+/? control	0.447 ± 0.013	1.04 ± 0.056	1.49 ± 0.058	0.79 ± 0.033	0.13 ± 0.022
cp/*cp* control	0.835 ± 0.043	1.86 ± 0.095	2.69 ± 0.136	2.41 ± 0.108	3.14 ± 0.344
cp/*cp* arginine silicate–treated	0.874 ± 0.022	1.84 ± 0.059	2.71 ± 0.079	2.30 ± 0.104	2.75 ± 0.276
cp/*cp* arginine HCl-treated	0.727 ± 0.046	1.60 ± 0.091	2.33 ± 0.136	1.97 ± 0.109 *	2.28 ± 0.160 *
Female rats					
+/? control	0.576 ± 0.015	1.46 ± 0.041	2.03 ± 0.052	1.10 ± 0.044	0.16 ± 0.026
cp/*cp* control	1.20 ± 0.069	1.74 ± 0.068	2.94 ± 0.060	3.56 ± 0.207	8.02 ± 0.576
cp/*cp* arginine silicate–treated	0.661 ± 0.043 ***	2.00 ± 0.062 *	2.66 ± 0.083 *	2.56 ± 0.147 ***	5.20 ± 0.473 **
cp/*cp* arginine HCl-treated	0.620 ± 0.062 ***	1.82 ± 0.080	2.43 ± 0.113 ***	2.44 ± 0.213 **	4.88 ± 0.740 **

Values are millimoles per liter; mean ± SEM, 10 rats in each group. Male rats were treated from 8 to 13 weeks of age and female rats from 12 to 19 weeks of age. Statistical differences are as indicated between groups. No statistical tests were made between +/? and *cp*/*cp* control groups as these are highly different.

* $P < .05$ vs *cp*/*cp* control.

** $P < .005$ vs *cp*/*cp* control.

*** $P < .001$ vs *cp*/*cp* control.

The physiological/metabolic role of silicon has not been widely studied. However, it is actively excreted in rats and may be an essential trace element [30]. There is evidence that silicon plays a significant role in both bone formation and immune function, with an interaction with arginine intake [31,32]. The *cp*/*cp* rats of both sexes had higher plasma silicon concentrations than the +/? rats, which we suggest is a consequence of the greater food intake of the obese hyperphagic animals. The plasma levels (2.0- and 1.37-fold higher in male and female *cp*/*cp* rats, respectively) are close to the food intakes relative to the +/? rats (1.65- and 1.38-fold higher). Our results show that the arginine silicate inositol complex is readily and directly absorbed in vivo, as indicated by the marked elevation of plasma silicon levels and the increased plasma arginine levels in the arginine silicate–treated rats. Mean plasma levels of arginine were lower in both male and female groups of rats treated with arginine HCl than in those treated with the arginine silicate, although the differences did not reach statistical significance. However, this consistent change, taken together with our previous finding of significant improvement in coronary artery relaxant function and reduction in glomerular sclerosis in the arginine silicate–treated rats, is suggestive of enhanced absorption and bioavailability of the silicate complex.

Similarly, only rats treated with arginine silicate showed significant changes in plasma NO_x levels, consistent with increased bioavailability of arginine and enhanced NO metabolism and the micro- and macrovascular effects previously reported. The opposite changes in NO_x levels observed in arginine-treated male and female *cp*/*cp* rats are apparently paradoxical. In male *cp*/*cp* rats, we speculate that the increased availability of arginine improved endothelial function and regulation of vascular contractility and thus reduced the overall net endothelial NO output required. This is consistent with the significant improvement in the more seriously impaired aortic vascular function of the male *cp*/*cp* rat [12]. Conversely, the rise in NO_x in the female rats implies a greater generation of NO from arginine in the presence of increased arginine availability, without a corresponding NO down-regulation. This paradox may reflect the very different status of the vascular system in male and female rats, with less marked vascular dysfunction in the *cp*/*cp* female rats [33] because of slower progression of the insulin resistance and more modest hyperinsulinemia compared with the *cp*/*cp* male rats [34]. The *cp*/*cp* female rats may also have differing responses to an increase in freely available arginine because of the altered activity of NOS, particularly of the eNOS isoform. It has been suggested by others that the limited efficacy of dietary arginine is due to the inhibitory action of asymmetric dimethyl arginine on eNOS or the inadequate intracellular concentrations of the cofactor tetrahydrobiopterin [10,11]. We have previously reported that impaired relaxation of the coronary vasculature of the male *cp*/*cp* rat is normalized by exogenous tetrahydrobiopterin [35], which suggests that inadequate tetrahydrobiopterin availability plays a role in the vascular dysfunction of the *cp*/*cp* male rat; and this can be compensated by increased arginine availability.

Arginine is a powerful insulin secretagogue, especially in the *cp*/*cp* rat [36]; and the increase in fasting insulin levels seen in the highly hyperinsulinemic male rats (Fig. 5) is most probably due to arginine-mediated insulin release. The absence of a greater insulin response to the meal tolerance test in the arginine-treated male *cp*/*cp* rats may simply reflect the already very high postprandial insulin output, making further insulin release impossible. In contrast, *cp*/*cp* female rats are less hyperinsulinemic and showed substantial reductions in insulin levels, both fasting and postprandial, and thus evidence of increased insulin sensitivity. These results suggest that arginine treatment may be more efficacious during early stages of, or in the presence of moderate rather than severe, insulin resistance.

Rats continue to gain weight throughout adult life, reaching an asymptotic weight in late middle age. It is noteworthy that whereas there was no weight reduction in male *cp*/*cp* rats, arginine silicate–treated female *cp*/*cp* rats showed a substantial reduction in the rate of weight gain and thus final body weight without any change in food intake.

There are numerous reports documenting that weight loss per se can improve insulin sensitivity. However, there is a paucity of understanding of whether improvements in insulin sensitivity (through arginine supplementation) can in turn induce weight loss, or at least reduce weight gain, as seen in this study. We know that female JCR:LA-*cp* rats have depressed levels of circulating estrogen as well as increased concentrations of testosterone [13,17]. We speculate that striking improvements to fasting and postprandial insulin in arginine-treated *cp*/*cp* female rats may indirectly elicit benefits to the hormonal axis and in turn cause a secondary modulation of weight gain. Concentrations of the adipocyte-derived peptide hormones leptin and adiponectin were not measured in this study and may prove to be useful in ongoing investigations.

There was a varied response of lipid parameters of *cp*/*cp* rats to arginine treatment, which may be explained (at least in part) by the large variance of values, particularly in triglyceride concentrations, that is characteristic of the *cp*/*cp* rat. Male *cp*/*cp* rats treated with arginine HCl, but not with arginine silicate, showed a significant reduction in triglyceride and phospholipid concentrations, consistent with a reduction in the hepatic hypersecretion of VLDL of the *cp*/*cp* rat [23,24]. One of the reviewers of this article has helpfully suggested that the absence of a significant reduction in VLDL secretion in the arginine silicate–treated male rats may be due to heterogeneity of the animals and that groups of 10 were thus underpowered. This is possible; but in the case of the triglyceride values, both the mean and variance were very little different between the *cp*/*cp* control and arginine silicate–treated groups, and there were no individual animals in the groups that could be considered outliers. In *cp*/*cp* female rats (which are inherently more overtly hypertriglyceridemic than *cp*/*cp* male rats), we observed a significant decrease in unesterified cholesterol, phospholipid, and triglyceride concentrations in those treated with both arginine preparations, indicative of a major reduction in VLDL production [37]. Moreover, we speculate that the small rise in cholesterol esters in the arginine silicate–treated female rats may also reflect a change in lipoprotein particle composition and shift to greater density and smaller-sized fractions (eg, to high-density lipoprotein). These changes may be expected to have favorable effects on cardiovascular disease risk and progression [38,39].

In summary, the present study, together with our previous report on vascular effects, demonstrates that a novel arginine silicate inositol complex raises circulating concentrations of arginine in a unique animal model of the metabolic syndrome and is more efficacious than arginine HCl. However, despite the comparable increase in arginine bioavailability in male and female JCR:LA-*cp* rats, we observed a sexual dimorphism in the metabolic response, suggesting that the potential beneficial effects of arginine supplementation may be related to hormonal status. Notably, treatment with arginine silicate was accompanied by striking beneficial effects in the female JCR:LA-*cp* rat, including improvements to insulin sensitivity, weight gain, and plasma lipids. This in turn may have implications for better understanding of sex-related insulin-resistance processes such as polycystic ovary syndrome. The marked improvement in end-stage micro- and macrovascular disease in the arginine silicate–treated *cp*/*cp* male rat reported earlier [12] occurred without improvement in insulin sensitivity, as we now report in the female rat. This suggests that enhanced NO metabolism has independent protective effects on the vascular system, probably at the level of the endothelium, that protect against the damaging effects of hyperinsulinemia. Our results indicate that the physiological and pathophysiological roles of arginine and silicon are complex and that adequate understanding will require significant further study. Collectively, the findings provide grounds for optimism that dietary supplementation with appropriate preparations of arginine may alleviate the metabolic profile typically associated with insulin resistance and associated metabolic syndrome(s).

Acknowledgments

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