Contrasting Effects of L-Arginine on Insulin-Mediated Blood Flow and Glucose Disposal in the Elderly

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Insulin increases skeletal muscle blood flow in healthy young subjects by a nitric oxide (NO)–dependent mechanism. Impairment of this mechanism may contribute to the insulin resistance of normal aging. We tested the hypothesis that L-arginine, the endogenous precursor for NO synthesis, would augment insulin-mediated vasodilation and in so doing increase insulin-mediated glucose uptake (IMGU) in healthy elderly subjects. Experiments were conducted on healthy young ($n = 9$; age, 24 ± 1 years; body mass index, 24 ± 1 kg/m^2) and old ($n = 9$; age, 77 ± 2 years; BMI, 25 ± 1 kg/m^2) subjects. Each underwent two euglycemic clamp studies. On both occasions, insulin was infused from 0 to 120 minutes (young, 40 mU/m^2/min; old, 34 mU/m^2/min). On 1 day, insulin was continued and L-arginine (7.5 mg/kg/min) was coinfused from 120 to 240 minutes. On the second study day, the insulin infusion from 120 minutes onward was adjusted in each subject to match corresponding plasma concentrations during the L-arginine infusion. Calf blood flow was measured bilaterally using venous occlusion plethysmography. Mean arterial blood pressure decreased in response to L-arginine in both young (77 ± 1 *v* 73 ± 1 mm Hg; $P < .05$) and old (103 ± 2 *v* 94 ± 2 mm Hg; $P < .01$). Calf vascular conductance increased in young (from 0.094 ± 0.009 to 0.113 ± 0.012 mL/100 mL/min/mm Hg; $P < .01$) and old (from 0.035 ± 0.003 to 0.050 ± 0.003 mL/100 mL/min/mm Hg; $P < .01$), consistent with the concept that the addition of substrate can augment skeletal muscle endothelial NO production in both age groups. Calf blood flow increased in both young (control, 7.04 ± 0.73; L-arginine, 8.02 ± 0.78 mL/100 mL/min; $P < .05$) and old (control, 3.60 ± 0.27: L-arginine, 4.65 ± 0.23 mL/100 mL/min; $P < .0001$) subjects, yet L-arginine had no impact on glucose disposal in either age group. In conclusion, L-arginine caused skeletal muscle vasodilation in the elderly, indicating that this endothelially mediated response is not attenuated with age. However, this increase in blood flow had no impact on insulin-mediated glucose uptake.

THE BULK OF insulin-mediated glucose uptake (IMGU) occurs in skeletal muscle. This may occur through several mechanisms. Initially, insulin was thought to promote glucose uptake into muscle primarily by activating glucose transporters. More recently it has been demonstrated that insulin can increase skeletal muscle blood flow via a nitric oxide (NO)–dependent mechanism.[1,2] However, whether insulin-mediated vasodilation is an important element of normal glucose disposal is controversial.[3,4] NO is synthesized from its natural precursor L-arginine in the endothelium via the enzyme NO synthase. Systemic infusion of L-arginine has been reported by some investigators to increase insulin-mediated blood flow and glucose disposal in healthy young subjects,[5] but its effects in healthy elderly subjects have not been described.

Normal aging is characterized by a progressive impairment in carbohydrate tolerance. One of the major factors contributing to the glucose intolerance of aging is resistance to IMGU.[6-9] Endothelial production of NO is reduced with age,[10] and it has recently been demonstrated that insulin-mediated vasodilation is impaired in the elderly.[11,12] We postulated that impaired endothelial production of NO in response to insulin causes this deficient insulin-mediated vasodilation, which in turn contributes to the insulin resistance of aging. If so, L-arginine would increase both insulin-mediated skeletal muscle blood flow and insulin-mediated glucose disposal in the elderly, thereby reversing their resistance to insulin. We undertook the present study to test this specific hypothesis.

SUBJECTS AND METHODS

Experimental Subjects

Studies were performed in healthy, nonobese young and elderly subjects (Table 1) recruited on the basis of normal findings on history and physical examination and normal laboratory test results (including hepatic and renal function, electrocardiogram, and glucose tolerance test, as defined by the National Diabetes Data Group criteria). No subject was taking medication. No subject had symptoms of claudication or signs of peripheral vascular insufficiency. All subjects had blood pressure and lipid values within the normal range for their age. Ankle blood pressure (as measured with a sphygmomanometer) was greater than or equal to arm blood pressure in all subjects. This protocol was approved by the Committee on Human Investigation at the University of British Columbia. All subjects provided written informed consent before participating.

Procedures

Each subject underwent two glucose clamp studies, each of 4 hours' duration, performed according to the method of Andres et al,[13] at least 2 weeks apart. Intravenous lines were inserted into an antecubital vein for infusion of substrates and into a contralateral hand vein for sampling of "arterialized" venous blood.[14] Blood pressure and heart rate were measured using an automated cuff method (Dinamap; Critikon

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Table 1. Subject Characteristics and Fasting Glucose and Hormone Levels

	Young (n = 9)	Old (n = 9)
Age (yr)	24 ± 1	77 ± 2
Male/female	5/4	5/4
BMI (kg/m^2)	24 ± 1	25 ± 1
MABP (mm Hg)	79 ± 2	101 ± 2*
Heart rate (beats/min)	55 ± 2	62 ± 2*
CBF (mL/100 mL/min)	4.46 ± 0.58	2.70 ± 0.12*
Glucose (mmol/L)	5.0 ± 0.1	5.2 ± 0.1
Insulin (pmol/L)	107 ± 15	106 ± 13

*$P < .01$, young *v* old.

Inc, Tampa, FL). Mean arterial blood pressure (MABP) was calculated as diastolic blood pressure plus one third of the pulse pressure.

Blood flow was determined simultaneously in both calves by venous occlusion plethysmography, using calibrated mercury-in-silastic strain gauges.[15] This technique was used because it has been shown to measure changes in calf blood flow in response to insulin infusion reliably.[16-19] Each leg was supported at 15 cm above the right atrium. Venous occlusion pressure was 40 mm Hg at the lower thigh, and ankle cuff occlusion pressure was 200 mm Hg. The occlusion cuff was inflated for 10 seconds and deflated for 10 seconds over a 3-minute period. The mean of the final five measurements of each recording period was used for analysis.

Protocol

After an overnight fast, studies began at 5:30 AM in our Clinical Research Centre. Once instrumentation was complete, baseline values were established over a 20-minute period (time −20 to 0 minutes). Three heparinized blood samples were taken at 10-minute intervals from −20 to 0 minutes to determine basal glucose and insulin levels. From 0 to 240 minutes, glucose was measured at 5-minute intervals and insulin every 30 minutes. Blood pressure was measured at baseline (−20 to 0 minutes), every 30 minutes from 0 to 120 minutes, and every 15 minutes from 120 to 240 minutes. Calf blood flow was measured at 10-minute intervals from −20 to 0 minutes, at 30-minute intervals from 0 to 120 minutes, and every 15 minutes for the duration of the study.

Glucose clamp studies began at time 0 and continued for 240 minutes. In the first (L-arginine) study, regular human insulin (Eli Lilly, Indianapolis, IN) was infused at a rate of 40 mU/m^2/min in the young and 34 mU/m^2/min in the old. From 120 minutes onward, L-arginine was coinfused with insulin in a nonprimed continuous manner at a rate of 7.5 mg/kg/min. On the second (control) study day, insulin was also infused at the above rates from 0 to 120 minutes, but from 120 minutes onward the infusion rate of insulin was adjusted in each subject to match the insulin concentrations observed during L-arginine infusion.

These insulin infusion rates were chosen because insulin clearance decreases with age.[20] They result in equivalent peripheral insulin levels in healthy young and elderly subjects.[11] When designing these experiments, we were concerned that stimulation of pancreatic insulin secretion by L-arginine might confound the interpretation of our findings. Because the effects of L-arginine on insulin secretion vary between subjects, we were obliged to perform the L-arginine study first. This allowed us to determine its effect on peripheral insulin levels and use this information to adjust the insulin infusion from 120 to 240 minutes of the second study to achieve comparable plasma insulin concentrations in each subject on both study days. On the L-arginine study day, two young and two elderly subjects underwent determination of peripheral glucose disposal and hepatic glucose production using tritiated glucose methodology.[21] The subjects and the technician performing the analysis of glucose levels, blood flow, and blood pressure measurements and responsible for the glucose clamp program were blinded as to the purpose and order of these studies.

Analytic Methods

Plasma glucose was measured immediately by the glucose oxidase method (YSI glucose analyzer; Yellowsprings Instruments, Yellow Springs, OH). The remaining blood was placed in prechilled test tubes containing Aprotonin (400 KIU/mL) and EDTA (1.5 mg/mL) and centrifuged at 4°C. The plasma was stored promptly at −70°C until assay. All samples from each subject were analyzed in the same assay. Insulin, glucagon, and growth hormone assays were performed as described previously.[11,22]

Data Analysis

Data are presented as mean ± standard error. To determine the effect of these interventions on substrate delivery to skeletal muscle, blood flows recorded simultaneously were averaged to provide a mean calf blood flow (CBF) value for each subject for each time point. To determine the effect of L-arginine on calf skeletal muscle endothelial NO synthase, calf vascular conductance (CVC) was calculated as the quotient of CBF and MABP.[23,24]

Measurements obtained during the last hour of the protocol (time 180 to 240 minutes) were considered steady-state values for the purpose of comparison between the L-arginine and the control study. Because L-arginine increased plasma insulin concentrations, we calculated the M/I ratio by dividing the glucose disposal rate at steady state by the insulin concentration at steady state.[25,26] Differences between groups were determined using Student's *t* test for paired or unpaired samples, as appropriate. $P < .05$ was considered significant.

RESULTS

Subject characteristics appear in Table 1. At baseline, the elderly had higher MABP and heart rates and lower CBF values. Body mass index (BMI) and fasting glucose and insulin values did not differ between the two groups (Table 1).

Effect of L-Arginine on Glucose Delivery to Skeletal Muscle and Uptake

Steady-state (180 to 240 minutes) glucose values were similar in young and old in both the control and L-arginine studies. In young subjects, plasma insulin concentrations at steady state were similar on both study days (control, 1,342 ± 152 pmol/L; L-arginine, 1,344 ± 93 pmol/L; P = NS). L-Arginine increased CBF from 7.04 ± 0.73 to 8.02 ± 0.78 mL/100 mL/min ($P < .05$) but had no effect on either glucose infusion rates (control, 9.86 ± 1.14 mg/kg/min; L-arginine, 11.15 ± 1.33 mg/kg/min; P = NS) or on M/I ratio (control, 0.010 ± 0.001; L-arginine, 0.011 ± 0.002 mg/kg/min/pmol/L; P = NS; Fig 1). In elderly subjects, plasma insulin concentrations at steady state were also similar on both study days (control, 1,248 ± 139 pmol/L; L-arginine, 1,271 ± 128 pmol/L; P = NS). As in young subjects, L-arginine increased CBF, from 3.60 ± 0.27 to 4.65 ± 0.23 mL/100 mL/min ($P < .0001$), but had no effect on either glucose infusion rates (control, 7.60 ± 0.33 mg/kg/min; L-arginine, 7.36 ± 0.40 mg/kg min; P = NS) or the M/I ratio (control, 0.006 ± 0.001; L-arginine, 0.006 ± 0.001 mg/kg/min/pmol/L; P = NS; Fig 1). The increase in CBF in response to insulin during the follow-up study was less in the aged (young, 2.64 ± 0.47; old, 1.03 ± 0.19 mL/100 mL/min; $P < .01$).

In two young and two old subjects, steady-state hepatic

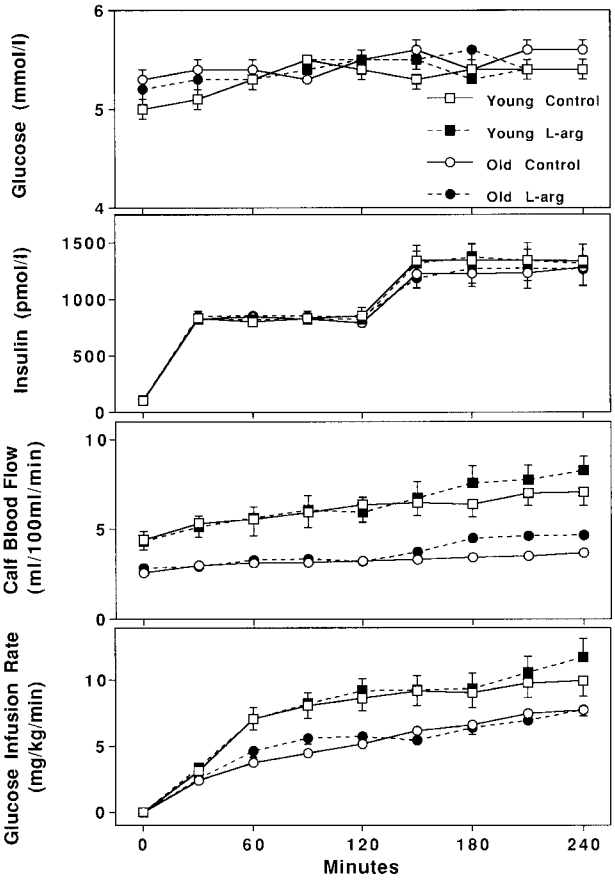

Fig 1. Glucose and insulin values, CBF, and glucose infusion rates in young and old subjects during the euglycemic clamp studies. CBF was greater with L-arginine in both young ($P < .05$) and elderly ($P < .0001$) subjects.

glucose production was suppressed to an equivalent degree in control (young, 0.10 ± 0.08; old, 0.12 ± 0.09 mg/kg/min; P = NS) and L-arginine (young, 0.12 ± 0.10; old, 0.15 ± 0.11 mg/kg/min; P = NS).

Effect of L-Arginine on Blood Pressure and Calf Vascular Conductance

In young subjects, L-arginine coinfusion decreased MABP from 77 ± 1 to 73 ± 1 mm Hg ($P < .05$) and increased both heart rate (from 62 ± 2 to 72 ± 2 beats/min; $P < .01$) and CVC (from 0.094 ± 0.009 to 0.113 ± 0.012, mL/100 mL/min/mm Hg; $P < .01$; Fig 2). Similar hemodynamic responses were observed in the elderly. L-Arginine coinfusion decreased MABP from 103 ± 2 to 94 ± 2 mm Hg ($P < .01$) and increased both heart rate (from 61 ± 2 to 69 ± 2 beats/min; $P < .01$) and CVC (from 0.035 ± 0.003 to 0.050 ± 0.003 mL/100 mL/min/mm Hg; $P < .0001$; Fig 2).

Effect of L-Arginine on Hormone Levels

In young subjects, L-arginine infusion increased both growth hormone (basal, 0.4 ± 0.1 μg/L; steady state, 15.4 ± 1.7 μg/L; $P < .0001$) and glucagon (basal, 62 ± 5 pg/mL; steady state, 172 ± 28 pg/mL; $P < .0001$) levels. There was no increase in growth hormone (basal, 0.8 ± 0.3 μg/L; steady state, 0.9 ± 0.4 μg/L; P = NS) during the control study, and glucagon levels decreased (basal, 75 ± 9 pg/mL; steady state, 36 ± 3 pg/mL; $P < .0001$). In elderly subjects, L-arginine infusion increased both growth hormone (basal, 0.9 ± 0.4 μg/L; steady state, 15.3 ± 2.8 μg/L; $P < .0001$) and glucagon (basal, 68 ± 4 pg/mL; steady state, 218 ± 11 pg/mL; $P < .0001$) levels. There was no increase in growth hormone (basal, 2.2 ± 1.0 μg/L; steady state, 4.5 ± 1.4 μg/L; P = NS) during the control study, and glucagon levels decreased (basal, 71 ± 7 pg/mL; steady state, 41 ± 3 pg/mL; $P < .0001$).

DISCUSSION

Healthy aging is characterized by reduced endothelial production of NO,[27,28] an attenuated effect of insulin on skeletal muscle blood flow, and resistance to IMGU. Several groups have demonstrated that insulin can stimulate blood flow in healthy young subjects by an NO-dependent mechanism.[1,2] It has been proposed that insulin-mediated vasodilation is an important component of its effect on glucose disposal, but this hypothesis is controversial.[3,4] In the present study, we evalu-

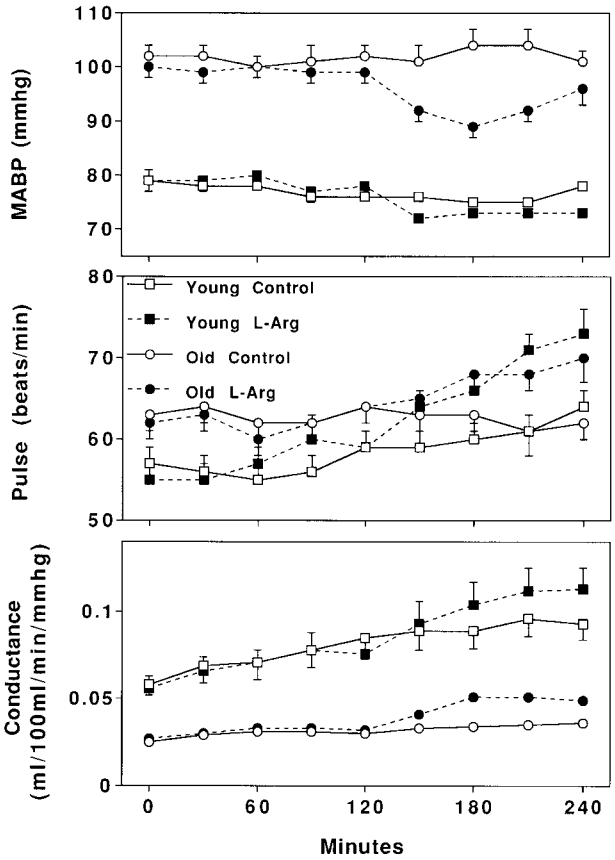

Fig 2. MABP, pulse rate, and CVC values in young and old subjects during the euglycemic clamp studies. L-Arginine infusion decreased MABP (young, $P < .05$; old, $P < .01$) and increased pulse rate (young, $P < .01$; old, $P < .01$) as well as CVC (young, $P < .01$; old, $P < .0001$) significantly in both age groups.

ated the effect of a systemic infusion of the NO precursor, L-arginine, on insulin-mediated blood flow and glucose disposal in healthy elderly subjects to test the hypothesis that L-arginine would overcome the age-related defect in insulin-mediated vasodilation and reverse the insulin resistance of aging. CBF was measured to determine the effect of this intervention on glucose delivery to skeletal muscle, whereas CVC, which reflects downstream resistance, was calculated to assess the effect of L-arginine on skeletal muscle endothelial NO production.[23,24]

There were two key findings in this study. First, coinfusion of L-arginine augmented the effect of insulin on CBF in the elderly, but this increase in glucose delivery to skeletal muscle did not result in a significant increase in glucose disposal or in the M/I ratio. This finding is consistent with the concept that increasing skeletal muscle blood flow does not affect insulin sensitivity significantly in healthy elderly subjects. Second, it has previously been reported that there is an age-related impairment of insulin-mediated vasodilation.[11,12] One potential mechanism for this effect may be deficient endothelial NO synthesis because decreased endothelial NO production in vitro[10] and a decrease in endothelium-dependent NO-mediated vasodilation in vivo[27,28] are reported to occur with normal aging. In the present study, coinfusion of L-arginine resulted in an increase in CVC and a decrease in MABP in both age groups, and the magnitude of these changes was similar. These observations in healthy elderly subjects are novel and indicate that the endothelium of healthy elderly subjects is capable of responding appropriately to increased delivery of this precursor, and that an age-related decrease in NO synthase cannot by itself account for the impaired insulin-mediated vasodilation and decreased NO production documented in this population.[27,28]

Other investigators have infused a variety of vasoactive agents into young healthy subjects to examine the effect of alterations in blood flow on insulin-mediated glucose disposal. Many studies have not shown concordance in the effects of these interventions on these two variables.[4] For example, systemic infusion of angiotensin II or norepinephrine vasoconstricts but enhances insulin-mediated glucose disposal.[29,30] In other experiments, femoral arterial infusion of metacholine, which stimulates endothelial production of NO, increased both leg blood flow and IMGU,[1,2] whereas bradykinin increased leg blood flow but had no effect on IMGU.[31,32] In previous experiments in our laboratory, the NO donor sodium nitroprusside increased insulin-mediated CBF but had no effect on insulin-mediated glucose disposal.[33] Thus, in healthy young subjects, the effects of insulin-mediated vasodilation on glucose disposal are uncertain.

To date, few studies have assessed the effect of L-arginine on insulin-mediated blood flow and glucose disposal in healthy young subjects.[5,34,35] Wascher et al[35] found that coinfusion of L-arginine with insulin enhanced insulin sensitivity but had no effect on insulin-mediated blood flow. Guigliano et al[5,34] reported that L-arginine infusion enhanced insulin-mediated vasodilation and insulin sensitivity. In one study, these investigators[34] concluded that the hemodynamic response was secondary to the increase in insulin concentrations stimulated by L-arginine rather than a direct vascular effect of this intervention. In a subsequent study, the same group of investigators concluded that L-arginine infusion resulted in a substantial increase in insulin-mediated vasodilation and glucose disposal independent of its effects on insulin levels.[5] In the latter report, they hypothesized that L-arginine enhanced insulin-mediated glucose uptake through an increase in peripheral blood flow as a result of increased endothelial NO production. The present observations are consistent with the concept that L-arginine enhances insulin-mediated vasodilation independent of its effects on insulin levels. However, despite increasing skeletal muscle blood flow, L-arginine had no significant effect on glucose disposal in the young. The reason for the discrepant results between studies is unclear but may relate to subject selection as well as differences in experimental protocols used during the studies.[3,4,34-36] In this regard, our subjects had a lower resting pulse rate than subjects in previous studies.[34,35] This implies that our subjects were more fit, which may partly explain the discrepancy. In addition, there was a trend toward increased glucose disposal with L-arginine in the young, and we may have detected a difference in glucose disposal rates if we had a larger sample size.

Several methodologic concerns should be addressed. It could be argued that it is inappropriate to imply that L-arginine's effects on insulin-mediated calf vasodilation are representative of its effects on skeletal muscle vasculature elsewhere in the body. The intent of this experiment was to evaluate specifically the effect of L-arginine on IMGU in the healthy elderly compared with healthy young subjects and relate this to L-arginine's action on insulin-mediated skeletal muscle vasodilation and vascular conductance. Study of the leg circulation is ideal for this purpose. The bulk of IMGU occurs in skeletal muscle, and the legs make up nearly 60% of skeletal muscle mass.[37,38] Previous studies have shown that there is a strong correlation between leg glucose uptake and whole-body glucose uptake.[39] In addition, vasodilation in response to insulin occurs selectively in skeletal muscle tissue and is comparable in the upper and the lower limbs.[40] Thus we believe it is reasonable to extrapolate findings in calf vasculature to skeletal muscle vasculature in general and to relate these findings to whole-body glucose disposal. Although levels of insulin were matched between control and L-arginine studies, C-peptide levels were undoubtedly higher during L-arginine infusion. Because C-peptide may stimulate blood flow by an NO-dependent mechanism, independent of insulin levels,[41] some of the effects of L-arginine on blood flow may have occurred because of stimulation of C-peptide secretion. L-arginine infusion is known to stimulate growth hormone and glucagon secretion,[42,43] and the levels of these hormones were higher during the L-arginine study. It is possible that increased levels of these hormones prevented us from demonstrating an increase in glucose disposal rates during L-arginine infusion because both glucagon and growth hormone are known to antagonize the effect of insulin on glucose uptake. We think this is unlikely because the effects of both of these hormones on glucose disposal generally take at least 2 hours to develop.[44,45] The absolute reduction in MABP during L-arginine infusion appeared to be greater in the elderly. It is possible that the discordance between the effects of L-arginine on substrate delivery and glucose disposal in the aged can be explained by an increase in sympathetic nervous

system activity in response to hypotension, which counteracted the effect of increased blood flow on IMGU. We think this explanation is unlikely because baroreflex sensitivity and responsiveness to catecholamines are impaired with age, and increases in limb vascular resistance during hypotensive stress are markedly reduced in older adults.[46,47] In addition, increased blood flow did not result in increased glucose uptake in the young, lending further support to the hypothesis that blood flow and glucose are not related in either age group.

Our protocol took into account several additional considerations. We infused insulin for 240 minutes because previous studies have suggested that insulin must be administered for several hours before its hemodynamic actions are fully manifest.[3,4,36] The L-arginine coinfusion was restricted to the last 2 hours to avoid nausea (which is a potent stimulus to the release of other vasoactive peptides). Indeed, in pilot studies of higher doses of L-arginine or more prolonged infusions, both young and old subjects experienced nausea. Furthermore, on the basis of previous reports, we anticipated that any confounding effects on blood flow or glucose disposal arising from increased pancreatic secretion of insulin stimulated by L-arginine would be delayed, whereas by comparison, its direct effects on blood flow and glucose disposal would occur relatively quickly.

In summary, systemic infusion of L-arginine decreased MABP and augmented the effect of insulin on CBF but did not increase insulin-mediated glucose disposal in the aged. The discordant action of L-arginine on CBF and glucose disposal suggests that the effect of insulin on glucose disposal is independent of its influence on skeletal muscle blood flow in the healthy elderly. Our finding of a consistent reduction in blood pressure and increases in insulin-mediated skeletal muscle blood flow raise the possibility that NO precursors have a role as adjunctive therapy in conditions characterized by endothelial dysfunction that commonly afflict the elderly, although this hypothesis must be tested in further studies. However, the present findings do not support the concept that administration of substrate with the intent of increasing NO synthesis will enhance insulin-mediated glucose disposal in elderly subjects with insulin resistance.

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