N(G)-Monomethyl-L-Arginine Alters Insulin-Mediated Calf Blood Flow But Not Glucose Disposal in the Elderly

Graydon S. Meneilly, Thomas Elliott, Bruno Battistini, and John S. Floras

It has been proposed that an important component of glucose disposal is insulin-mediated vasodilation via a nitric oxide (NO)-dependent mechanism. Normal aging is characterized by a resistance to insulin-mediated glucose disposal and deficient endothelial NO production. Impairment of insulin-mediated vasodilation could contribute to this insulin resistance. We tested the hypothesis that the NO synthase inhibitor *N*(G)-monomethyl-L-arginine (L-NMMA) would decrease insulin-mediated calf vasodilation and whole-body glucose disposal in young subjects but would have little or no effect in the elderly. Experiments were performed on healthy young (n = 10) and old (n = 10) subjects on 2 study days. Insulin was infused for 4 hours at 40 mU/m^2/min (young) and 34 mU/m^2/min (old) during both studies, and L-NMMA (0.1 mg/kg/min) was coinfused during the last 2 hours of insulin on one of these sessions. Calf blood flow was measured by venous occlusion plethysmography, and calf vascular conductance was derived from calf blood flow and mean arterial blood pressure (MABP). L-NMMA increased whole-body insulin-mediated glucose uptake (IMGU) in young subjects (from 11.22 ± 0.08 to 12.22 ± 0.87 mg/kg/min, *P* < .05) but decreased calf blood flow (from 6.53 ± 0.62 to 5.49 ± 0.43 mL/100 mL/min, *P* < .05). In contrast, L-NMMA had no effect on IMGU in elderly subjects (control *v* L-NMMA, 7.58 ± 0.46 *v* 7.86 ± 0.37 mg/kg/min, *P* = nonsignificant) but increased calf blood flow (from 3.65 ± 0.36 to 4.50 ± 0.32 mL/100 mL/min, *P* < .01). L-NMMA decreased calf vascular conductance in young subjects (from 0.083 ± 0.008 to 0.064 ± 0.005 mL/100 mL/min/mm Hg, *P* < .05) but not in the elderly (control *v* L-NMMA, 0.038 ± 0.004 *v* 0.040 ± 0.002 mL/100 mL/min/mm Hg), consistent with the concept that skeletal muscle endothelial NO production is reduced with age. We therefore conclude that (1) L-NMMA has different or opposite actions on calf blood flow and IMGU in both age groups, indicating that the effect of insulin on skeletal muscle blood flow is independent of its influence on glucose disposal in young and old, and (2) skeletal muscle NO production decreases with age.

INSULIN-MEDIATED GLUCOSE UPTAKE (IMGU) occurs primarily in skeletal muscle. This may be due to several mechanisms. Initially, insulin was considered to promote glucose uptake into muscle principally by activating glucose transporters. Insulin also increases skeletal muscle blood flow via a nitric oxide (NO)-dependent mechanism,[1,2] but whether insulin-mediated vasodilation is an important component of glucose disposal in healthy young subjects is uncertain.[1,2]

Normal aging is characterized by a progressive impairment in carbohydrate tolerance. One of the major contributing factors to the glucose intolerance of aging is a resistance to IMGU.[3-6] Endothelial production of NO is reportedly reduced with age.[7] We have recently demonstrated that insulin-mediated vasodilation is impaired in the elderly.[8] To explain these results, we postulated that impaired endothelial production of NO in response to insulin causes this defect in insulin-mediated vasodilation with aging, which in turn contributes to insulin resistance.

These observations prompted us to undertake the present study to test the hypotheses that (1) the NO synthase inhibitor *N*(G)-monomethyl-L-arginine (L-NMMA) would reduce insulin-mediated calf blood flow and whole-body glucose disposal in healthy young subjects, and (2) L-NMMA would have little or no effect on insulin-mediated calf blood flow or IMGU in the elderly. If so, this would be consistent with the concept that endothelial production of NO in skeletal muscle decreases with age.

SUBJECTS AND METHODS

Experimental Subjects

Studies were performed in healthy non-obese young and elderly subjects (Table 1) recruited on the basis of a normal medical history and physical examination, normal laboratory tests (including hepatic and renal function), a normal electrocardiogram, and a normal glucose tolerance test as defined by the National Diabetes Data Group criteria. None of the subjects were using medication. No subjects had symptoms of claudication. The pedal pulses were normal and there were no clinical signs of peripheral vascular insufficiency. All elderly subjects had baseline systolic and diastolic blood pressure less than 160 and less than 100 mm Hg, respectively. The ankle blood pressure (as measured with a sphygmomanometer) was greater than or equal to the arm blood pressure in all subjects. Lipid values in all elderly subjects were within the normal range for their age (cholesterol, 5.1 ± 0.2 mmol/L; low-density lipoprotein, 3.2 ± 0.2 mmol/L; high-density lipoprotein, 1.1 ± 0.1 mmol/L; triglyceride, 1.3 ± 0.3 mmol/L). The study was approved by the Committee on Human Investigation at the University of British Columbia. All subjects provided written informed consent prior to participation.

Experimental Protocol

Each subject underwent 2 glucose clamp studies in random order according to the method of Andres et al[9,10] and separated by at least 2

From the Divisions of Geriatric Medicine and Endocrinology, Department of Medicine, University of British Columbia, Vancouver, British Columbia; Quebec Heart and Lung Institute, Laval Hospital, Department of Medicine, Laval University, Ste-Foy, Quebec; and Division of Cardiology, Department of Medicine, University of Toronto, Toronto, Ontario, Canada.

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Address reprint requests to Graydon S. Meneilly, MD, Room S169, Vancouver Hospital and Health Sciences Centre, UBC Site, 2211 Wesbrook Mall, Vancouver BC, V6T 2B5.

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Table 1. Subject Characteristics and Fasting Glucose and Hormone Levels

Paramter	Young (n = 10)	Old (n = 10)
Age (yr)	23 ± 1	78 ± 2
Male/female ratio	6/4	5/5
BMI (kg/m^2)	23 ± 1	25 ± 1
MABP (mm Hg)	78 ± 2	96 ± 1*
Pulse rate (bpm)	56 ± 2	58 ± 1
Calf blood flow (mL/100 mL/min)	3.74 ± 0.25	2.72 ± 0.19*
Glucose (mmol/L)	5.0 ± 0.1	5.2 ± 0.1
Insulin (pmol/L)	103 ± 17	98 ± 12

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

weeks. Studies commenced at 7:30 AM after an overnight fast. Three heparinized blood samples were taken at 10-minute intervals from −20 to 0 minutes to measure basal glucose and insulin levels. At time 0, glucose clamp studies were commenced and continued for 240 minutes. In each 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. In the control study, insulin and glucose alone were infused. In the L-NMMA study, we infused L-NMMA (Clinalfa, Weidenmattweg, Switzerland), a stereospecific inhibitor of NO synthase, from 120 to 240 minutes at a rate of 0.1 mg/kg/min. Blood samples were taken at 5-minute intervals to measure plasma glucose levels and every 30 minutes to measure insulin levels in each study. The total amount of blood withdrawn in each study was less than 90 mL. The coefficient of variation of plasma glucose did not exceed 5% in any study. Two young and 2 old subjects underwent a determination of peripheral glucose disposal and hepatic glucose production using tritiated-glucose methodology as previously described.[11]

The blood pressure and pulse were measured at baseline, every 30 minutes from 0 to 120 minutes, and every 15 minutes from 120 to 240 minutes using an automated blood pressure cuff (Dinamap, Critikon, Tampa, FL). Mean arterial blood pressure (MABP) was calculated from the diastolic blood pressure plus one third of the pulse pressure. Bilateral calf blood flow was determined by venous occlusion plethysmography using calibrated mercury-in-silastic strain gauges as previously described.[8] 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 rest of the study.

Analytic Methods

The plasma glucose level was measured immediately in all studies by the glucose oxidase method on a YSI glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH). Insulin assays were performed as previously described.[8]

Data Analysis

The data are presented as the mean ± SE. Calf vascular conductance was calculated as the quotient of calf blood flow and MABP.[12,13] 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-NMMA study and the control study. We calculated the M/I ratio by dividing the glucose disposal rate at steady state by the insulin value at steady state.[14,15] Differences between groups were determined using Student's *t* test for paired or unpaired samples as appropriate. A *P* value less than .05 was considered significant.

RESULTS

Subject characteristics are shown in Table 1. At baseline, the elderly had a higher MABP and lower calf blood flow. The body mass index (BMI), pulse rate, and fasting glucose and insulin values were not different between the groups.

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

In young subjects, the addition of L-NMMA resulted in an increase in the steady-state glucose infusion rate (from 11.22 ± 0.88 to 12.22 ± 0.87 mg/kg/min, $P < .05$; Fig 1). However, because L-NMMA also increased plasma insulin at steady state (from 770 ± 46 to 870 ± 53 pmol/L, $P < .01$; Fig 1), the M/I ratio was calculated. This ratio was identical on the 2 study days (control *v* L-NMMA, 0.016 ± 0.002 *v* 0.016 ± 0.002 mg/kg/min/pmol/L, P = NS). L-NMMA coinfusion decreased steady-state calf blood flow from 6.53 ± 0.62 to 5.49 ± 0.43 mL/100 mL/min (control *v* L-NMMA, $P < .05$; Fig 2).

In elderly subjects, L-NMMA coinfusion did not decrease the steady-state glucose infusion rate (control *v* L-NMMA, 7.58 ± 0.46 *v* 7.86 ± 0.37 mg/kg/min, P = NS; Fig 1). L-NMMA increased plasma insulin in these elderly subjects as well (from 776 ± 35 to 880 ± 34 pmol/L, $P < .001$; Fig 1), but their M/I ratio was again identical on the 2 study days (control *v* L-NMMA, 0.010 ± 0.001 *v* 0.009 ± 0.001 mg/kg/min/pmol/L, P = NS). In contrast to the young subjects, steady-state calf blood flow increased from 3.65 ± 0.36 to 4.50 ± 0.32 mL/100 mL/min (control *v* L-NMMA, $P < .01$; Fig 2).

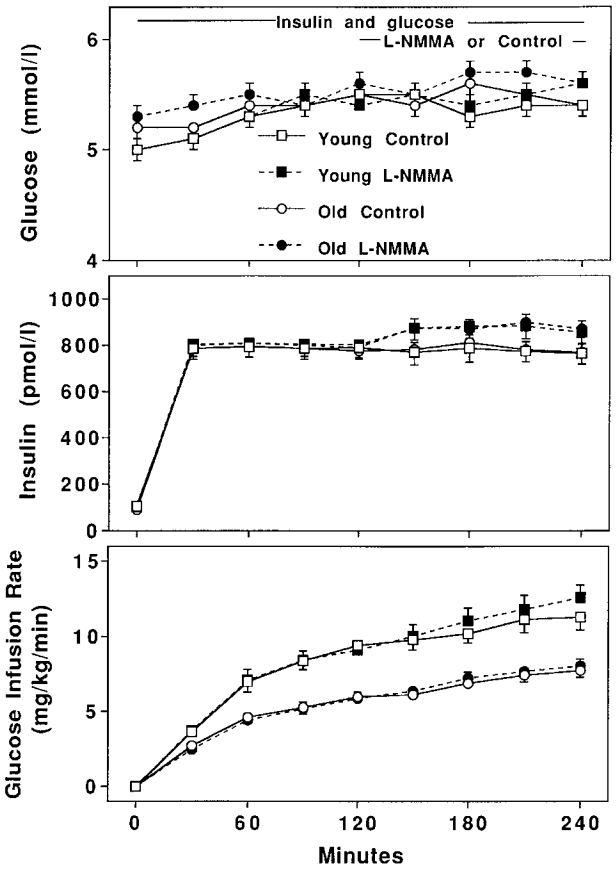

Fig 1. Glucose and insulin values and glucose infusion rate in young and old subjects during the euglycemic clamp studies.

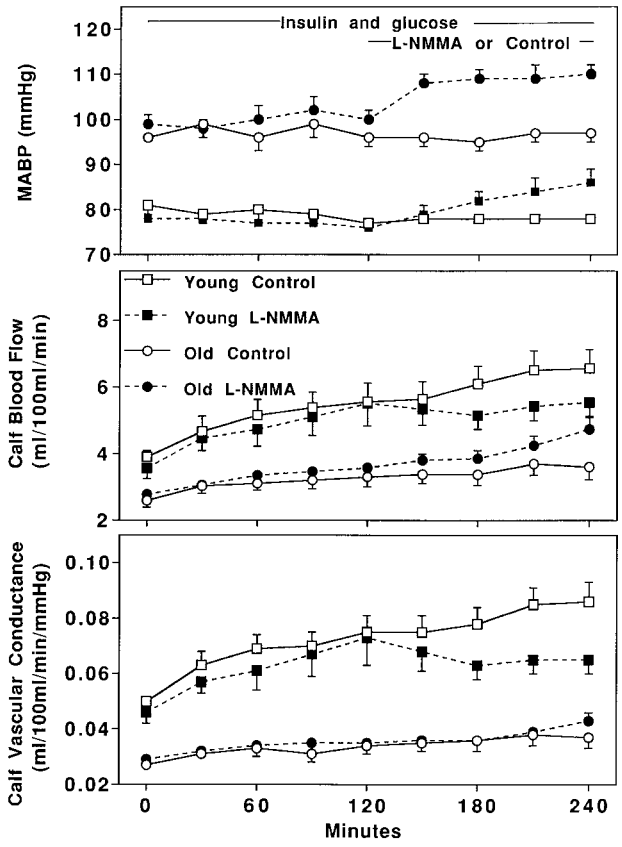

Fig 2. MABP, calf vascular conductance, and calf blood flow in young and old subjects during the euglycemic clamp studies.

Steady-state glucose values on the corresponding study days were similar in young and old subjects (Fig 1). Hepatic glucose uptake was suppressed to less than 0.5 mg/kg/min on both study days in the 2 young and 2 old subjects in whom it was determined.

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

Hemodynamic parameters are shown in Fig 2. In young subjects, L-NMMA coinfusion increased MABP by 9% ± 2% from 78 ± 1 to 85 ± 3 mm Hg ($P < .01$). The heart rate decreased from 64 ± 2 to 53 ± 3 bpm ($P < .001$). Calf vascular conductance at steady state (180 to 240 minutes) was significantly lower on the L-NMMA study day (0.064 ± 0.005 mL/100 mL/min/mm Hg) versus the control day (0.083 ± 0.008 mL/100 mL/min/mm Hg, $P < .05$), consistent with the concept that L-NMMA caused significant inhibition of skeletal muscle NO synthase.

In the elderly, L-NMMA coinfusion increased MABP by 13% ± 3% (P = NS *v* young subjects) from 97 ± 2 to 110 ± 2 mm Hg ($P < .0001$). The heart rate decreased from 62 ± 3 to 56 ± 2 bpm ($P < .0001$). However, in contrast to young subjects, L-NMMA had no effect on steady-state calf vascular conductance (control *v* L-NMMA, 0.038 ± 0.004 *v* 0.040 ± 0.002 mL/100 mL/min/mm Hg, P = NS). This observation is consistent with the concept that skeletal muscle endothelial NO production is reduced with normal aging.

Additional Comparisons

In the control study, there was a significant increase over basal in calf blood flow in response to insulin in both age groups (young, 2.68 ± 0.49 mL/100 mL/min, $P < .0001$; old, 0.74 ± 0.23 mL/100 mL/min, $P < .001$). However, the increment in calf blood flow in response to insulin was significantly less in the elderly ($P = .002$). In addition, the change in calf blood flow in response to L-NMMA was significantly different between young and old (young *v* old, −1.09 ± 0.33 *v* 0.84 ± 0.27 mL/100 mL/min, $P < .0001$).

DISCUSSION

These experiments resulted in two novel findings. First, L-NMMA had discordant effects on substrate delivery and glucose disposal in skeletal muscle (ie, calf blood flow) in both young and old subjects. That this occurred in both groups indicates that the effect of insulin on skeletal muscle blood flow is independent of its influence on glucose disposal in both age groups. Second, L-NMMA reduced calf vascular conductance significantly in young subjects, but had no effect in the elderly. This observation is consistent with the concept that skeletal muscle endothelial NO production decreases with age.

Previous investigators have infused a variety of vasoactive agents into young healthy subjects to examine the effect of increases in blood flow on insulin-mediated glucose disposal. Few of these studies have shown concordance in the effects of these interventions on the two variables.[1,2,16-22] In these studies, we elected to take the opposite approach and assess the effect of inhibition of NO production on blood flow and glucose disposal by administering the NO synthase inhibitor L-NMMA. Only one previous study has evaluated the effect of systemic L-NMMA infusion on insulin-mediated glucose disposal and blood flow in healthy young subjects. Butler et al[23] infused L-NMMA intravenously and found an increase in both calf blood flow and insulin sensitivity. Although the increase in the glucose disposal rate they report is consistent with the present findings, the mechanism for paradoxic vasodilation in response to L-NMMA is unclear. In the present experiments, we infused double the dose of L-NMMA and observed the decrease in calf vascular conductance and calf blood flow anticipated in healthy young subjects. Despite this decrease in substrate delivery, we did not detect a corresponding reduction in IMGU.

Normal aging is characterized by a resistance to insulin-mediated glucose disposal.[3-6] It has been assumed that insulin resistance in the elderly is due to a postreceptor defect in insulin action. Although early studies did not detect an aging effect on insulin-mediated blood flow,[24] our group and others recently demonstrated that insulin-induced vasodilation is indeed impaired in the elderly.[8,25] Consequently, we postulated that impaired insulin-mediated vasodilation is an important cause of the insulin resistance of aging. One mechanism underlying this age-related impairment of insulin-mediated vasodilation may be deficient endothelial NO synthesis. Decreased endothelial NO production in vitro[7] and a decrease in endothelium-dependent and NO-mediated vasodilation in vivo[26,27] are

reported to occur with normal aging. Consistent with the concept that skeletal muscle endothelial NO production is reduced with normal aging, L-NMMA had no effect on calf vascular conductance in the elderly. L-NMMA resulted in an increase in calf blood flow and hence substrate delivery to this limb. Despite this, there was no corresponding increase in glucose uptake, which was similar on the 2 study days. This indicates that the effect of insulin on skeletal muscle blood flow is independent of its influence on glucose disposal in the elderly.

The infusion of L-NMMA resulted in a similar relative increase in blood pressure in young and old. There are several potential mechanisms that could account for these systemic effects, but these are beyond the scope of the present experiments. Suffice it to say that L-NMMA is not a direct vasoconstrictor, and its vascular effects arise from withdrawal of the tonic vasodilator actions of endothelially produced NO, thereby altering the local balance between endogenous pressor and depressor systems. To address this issue, further studies are needed to clarify the local and systemic vascular effects of L-NMMA in elderly subjects, and these studies will require the infusion of L-NMMA into different vascular beds.

Several methodologic concerns should be addressed. Hypertension is characterized by both insulin resistance and impaired NO availability.[1,2] Our findings could be due to hypertension and not normal aging. This is unlikely, because normal aging is characterized by a gradual increase in systolic blood pressure, so it is anticipated that normal elderly subjects would have higher systolic and mean blood pressure than a young control group. All of our subjects had baseline systolic blood pressure within the normal range for their age. Hyperlipidemia can affect endothelial function.[1] This is unlikely to have altered our results, since all of our elderly subjects had lipid values within the normal range. Insulin levels were higher during the L-NMMA study versus the control study in both age groups. To correct for these higher plasma concentrations, we calculated the M/I ratio, which has been shown to be a reliable measure of insulin sensitivity when insulin values differ.[14,15] It is possible that changes in calf blood flow were not due to insulin but were related to differences in the metabolic rate of glucose. This is unlikely, since previous studies have shown that it is hyperinsulinemia and not insulin-induced stimulation of carbohydrate metabolism that is the main mechanism for vasodilation in skeletal muscle.[28]

In summary, skeletal muscle endothelial NO production is reduced with normal aging, but this change cannot account for the impairment of glucose uptake in healthy elderly subjects. Rather, the discordant actions of L-NMMA on calf blood flow and IMGU in both groups indicate that the effect of insulin on skeletal muscle blood flow is independent of its influence on glucose disposal in youth and with age.

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