JCI The Journal of Clinical Investigation

NG-monomethyl-L-arginine inhibits the blood flow but not the insulin-like response of forearm muscle to IGF- I: possible role of nitric oxide in muscle protein synthesis.

D A Fryburg

J Clin Invest. 1996;97(5):1319-1328. https://doi.org/10.1172/JCI118548.

Research Article

In human skeletal muscle, insulin-like growth factor-I (IGF-I) exerts both growth hormone-like (increase in protein synthesis) and insulin-like (decrease in protein degradation and increase in glucose uptake) actions and augments forearm blood flow two- to threefold. This study was designed to address whether (a) the increase in blood flow due to IGF-I could be blocked by an inhibitor of nitric oxide synthase; and (b) the metabolic actions of IGF-I were altered by use of a nitric oxide synthase inhibitor. Forearm blood flow, glucose, lactate, oxygen, nitrite, and phenylalanine balances and phenylalanine kinetics were studied in a total of 17 healthy, adult volunteers after an overnight fast in two different protocols. In protocol 1, after basal samples IGF-I was infused alone for 4 h with samples repeated during the last 30 min. After the 4-h sample period, NG-monomethyl-L-arginine (L-NMMA) was infused into the brachial artery for 2 h to bring flow back to baseline and repeat samples were taken (6 h). In response to IGF-I alone, forearm blood flow rose from 3.8 +/- 1.0 (bas) to 7.9 +/- l.9 (4 h) ml/min/100 ml ($P < 0.01$) and was reduced back to baseline by L-NMMA at 6 h ($P < 0.01$). In protocol 1, IGF-I alone increased forearm nitrite release at 4 h ($P < 0.03$), which was reduced back to baseline […]

N^G-monomethyl-L-arginine Inhibits the Blood Flow but Not the Insulin-like Response of Forearm Muscle to IGF-I

Possible Role of Nitric Oxide in Muscle Protein Synthesis

David A. Fryburg
Division of Endocrinology and Metabolism, Department of Internal Medicine, and the General Clinical Research Center, University of Virginia Health Sciences Center, Charlottesville, Virginia 22908

Abstract

In human skeletal muscle, insulin-like growth factor-I (IGF-I) exerts both growth hormone–like (increase in protein synthesis) and insulin-like (decrease in protein degradation and increase in glucose uptake) actions and augments forearm blood flow two- to threefold. This study was designed to address whether (*a*) the increase in blood flow due to IGF-I could be blocked by an inhibitor of nitric oxide synthase; and (*b*) the metabolic actions of IGF-I were altered by use of a nitric oxide synthase inhibitor.

Forearm blood flow, glucose, lactate, oxygen, nitrite, and phenylalanine balances and phenylalanine kinetics were studied in a total of 17 healthy, adult volunteers after an overnight fast in two different protocols. In protocol 1, after basal samples IGF-I was infused alone for 4 h with samples repeated during the last 30 min. After the 4-h sample period, N^G-monomethyl-L-arginine (L-NMMA) was infused into the brachial artery for 2 h to bring flow back to baseline and repeat samples were taken (6 h). In response to IGF-I alone, forearm blood flow rose from 3.8±1.0 (bas) to 7.9±1.9 (4 h) ml/min/100 ml ($P < 0.01$) and was reduced back to baseline by L-NMMA at 6 h ($P < 0.01$). In protocol 1, IGF-I alone increased forearm nitrite release at 4 h ($P < 0.03$), which was reduced back to baseline by L-NMMA at 6 h ($P < 0.05$). Despite the reduction in flow with L-NMMA, IGF+L-NMMA yielded increases in glucose uptake ($P < 0.005$), lactate release ($P < 0.04$), oxygen uptake ($P < 0.01$), and a positive shift in phenylalanine balance ($P < 0.01$) due to both an increase in muscle protein synthesis ($P < 0.02$) and a decrease in protein degradation ($P < 0.03$).

In protocol 2, L-NMMA was coinfused with IGF-I for 6 h, with the dose titrated to keep blood flow ±25% of baseline. Coinfusion of L-NMMA restrained blood flow to baseline and also yielded the same, significant metabolic effects, except that no significant increase in muscle protein synthesis was detected. These observations suggest: (*a*) that IGF-I increases blood flow through a nitric oxide–dependent mechanism; (*b*) that total blood flow does not affect the insulin-like response of muscle to IGF-I; and (*c*) that nitric oxide may be required for the protein synthetic (growth hormone–like) response of muscle to IGF-I. (*J. Clin. Invest.* 1996. 97: 1319–1328.) Key words: phenylalanine • nitrate • nitrite • oxygen consumption • protein degradation

Introduction

It has long been recognized that increases in skeletal muscle metabolic activity are associated with increases in blood flow to muscle (1). Although it has been suggested that the increase in blood flow is driven by skeletal muscle to support increased metabolic demand (1, 2), it nonetheless has been difficult to separate the contribution of blood flow, per se, to the observed change(s) in muscle metabolism. The potential importance of this issue has been underscored by recent studies examining the relationship between insulin action and the insulin-induced increase in blood flow (3–5). As observed in normal subjects, in insulin-resistant patients the metabolic (glucose uptake) response to insulin is closely correlated with the blood flow response to insulin (3–5). These studies have suggested that the increase in flow may be important to promote or facilitate the uptake of hormone and substrate by muscle.

In the human forearm, insulin-like growth factor-I (IGF-I) markedly accelerates blood flow (6, 7). These changes in forearm blood flow are accompanied by both growth hormone–like (stimulation of protein synthesis) as well as insulin-like actions (decreases in protein degradation and increases in glucose uptake and lactate release) in forearm muscle (6). As recent observations have shown that insulin increases forearm or leg blood flow through a nitric oxide–dependent mechanism (8, 9) and that IGF-I increases nitric oxide production in vitro (10), the present studies were designed to examine (*a*) if the increase in blood flow due to IGF-I is also nitric oxide dependent; and (*b*) the relationship among nitric oxide synthase inhibition, blood flow, and the expression of IGF-I's metabolic actions.

Methods

Subjects

17 healthy, normal weight, adult volunteers, 25±5 yr of age, were admitted to the University of Virginia General Clinical Research Center the evening before study. Eight of these subjects participated in protocol 1 and nine participated in protocol 2. No subject was taking any medication and all female participants had a negative serum pregnancy test 1–2 d before study. The study protocol was approved by the University of Virginia Human Investigation Committee and each subject gave written consent.

Address correspondence to David A. Fryburg, M.D., Division of Endocrinology and Metabolism, Department of Internal Medicine, University of Virginia Health Sciences Center, Box 5116, MR4, Charlottesville, VA 22908. Phone: 804-924-1175; FAX: 804-924-1284; E-mail: daf2s@virginia.edu.

Received for publication 21 September 1995 and accepted in revised form 8 December 1995.

J. Clin. Invest.

0021-9738/96/03/1319/10 $2.00
Volume 97, Number 5, March 1996, 1319–1328

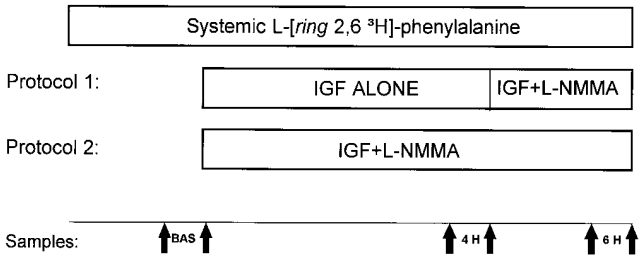

Figure 1. Experimental paradigms. Each subject had a systemic infusion of [^{3}H]phenylalanine begun 90 min before the basal (*BAS*) sample sets as described in Methods. In protocol 1, between 3.5 and 4 h of IGF-I alone, repeat samples were drawn from both arms (*4 H*). After the 4-h sample set, L-NMMA was coinfused with IGF-I for the next 2 h, the dose titrated to return forearm blood flow to baseline. Repeat samples were drawn at 6 h. For protocol 2, the L-NMMA infusion was initiated with IGF-I immediately after BAS samples. Samples were acquired at the same time points as in protocol 1.

Experimental protocol

Two different protocols were used, which are schematically depicted in Fig. 1. In both protocols, after an overnight 12-h fast, a brachial artery and an ipsilateral, retrograde, median cubital (deep) vein catheter were placed percutaneously. In the contralateral arm, a second, retrograde, median cubital vein catheter was placed. Each subject received a primed ($\sim$ 33 μCi), continuous (0.43 μCi/min) infusion of L-[*ring* 2,6-^{3}H]phenylalanine through a catheter placed in the lower extremity. After a 90-min tracer equilibrium period, quadruplicate, paired arterial and bilateral venous samples were taken for glucose, lactate, oxygen, and phenylalanine concentrations and phenylalanine specific activities from the arterial and each forearm venous catheter. Samples for IGF-I were taken from both deep veins. Forearm blood flow was measured in each arm after each set of arterial and venous samples by capacitance plethysmography.

Protocol 1. After obtaining baseline samples, IGF-I (Genentech Inc., South San Francisco, CA) was continuously infused into the brachial artery at 6.0 μg/kg bwt/min for 6 h. For the first 4 h of the IGF-I infusion, blood flow was measured every 30 min. During the last 30 min of this 4-h period (330–360 min), repeat, quadruplicate blood samples as described for the baseline period were acquired (samples designated IGF alone). After this middle sample set and for the last 2 h of the IGF-I infusion, N^G-monomethyl-L-arginine (L-NMMA)[1] (Novabiochem USA, La Jolla, CA) was coinfused into the brachial artery. The L-NMMA infusion was initiated at a dose of 25 nmol/kg bwt/min and was diluted in 0.9% saline. Blood flow was measured every 15 min. If, after two consecutive flow measurements, flow was $>$ 25% above baseline, the L-NMMA infusion was increased by 25 nmol/kg bwt/min. The maximal dose of L-NMMA infused was 100 nmol/kg bwt/min. Repeat measurements over 30 min as described for the basal and IGF periods were made at 6 h (450–480 min) since the infusions began. Blood pressure and heart rate were monitored during the study.

Protocol 2. This protocol was similar to protocol 1, except that L-NMMA infusion was initiated simultaneously with IGF-I. The dose at initiation was 25 nmol/kg bwt/min. Blood flow was measured every 15 min through the entire 6 h of the IGF/L-NMMA infusion. As described above, if blood flow rose $>$ 25% over baseline in two consecutive readings, then the L-NMMA infusion was stepped up by 25 nmol/kg bwt/min. No subject received $>$ 100 nmol/kg bwt/min. As described in protocol 1, repeat samples were drawn after 4 h and then 6 h of the combined infusion. For both protocols 1 and 2, the L-NMMA infusion rates were not changed during the sampling periods. Blood pressure and heart rate were monitored during the entire study.

Analytic methods

Blood flow was measured in both arms at the bedside using venous capacitance plethysmography (UFI, Morro Bay, CA). Like other venous plethysmography methods, this technique uses the change in volume of the forearm after brief venous outflow obstruction above the antecubital fossa. With capacitance plethysmography, the change in volume caused by venous outflow obstruction distorts the capacitor network wrapped around the midforearm and yields an electrical signal, which, by use of a predefined calibration signal, is then converted into a volume measurement (11, 12). Practically, 2 min after a wrist cuff was inflated to 200 mmHg to obstruct flow to and from the hand, a stable baseline tracing was obtained. Two calibration spikes were recorded and the venous outflow (supraantecubital) cuff was inflated to 40 mmHg. The recording was continued until the tracing plateaued. Blood flow rates were calculated from the rate of change in volume (milliliters per minute), divided by the volume under the capacitance network cuff. All flows were measured and results were calculated by the same observer. This method correlates well with the indocyanine green dye-dilution method (reference 12 and Fryburg, D., unpublished results).

Whole blood glucose and lactate concentrations were measured by a combined glucose/lactate analyzer (Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin and IGF-I (after acid ethanol extraction) were measured by radioimmunoassay. Phenylalanine concentration and specific activity were measured by ion pair, reverse-phase HPLC, as described previously (13). Percent oxygen saturation of blood and hemoglobin concentrations was determined with a Radiometer OSM hemoximeter (Radiometer, Copenhagen, Denmark).

Total plasma nitrate and nitrite were measured in protocol 1 using modifications of the methods of Tracey (14) and Gilliam et al. (15). This method converts nitrate to nitrite and then assays the nitrite using a well accepted colorimetric method. Briefly, to a solution of K_2HPO_4 (500 μl, 0.14 M), FAD (25 μl, 100 μM), NADPH (8 μl of 12.5 mM), and nitrate free H_2O, 250 μl of plasma was added after spinning at 4,500 rpm for 10 min. Nitrate reductase (*Aspergillus sp.*) was hydrated with K_2HPO_4 buffer for 30 min at room temperature before use (activity = 3.5 U/ml). After hydration, 17 μl of nitrate reductase was added to each sample, vortexed, and then incubated for 3 h in the dark (total volume = 1.150 ml). Using this method, the efficiency of conversion of nitrate to nitrite in plasma is $>$ 95% on average, as estimated by the recovery of nitrite from samples spiked with graded amounts of nitrate. After the incubation period, pyruvate (10 μl of 1.0 M stock) and lactate dehydrogenase (10 μl, stock concentration 938 U/ml) were added to each sample and mixed and warmed in a water bath at 37°C for 5 min. After the addition of lactate dehydrogenase and pyruvate (to remove excess NADPH), 0.5 ml of this mixture was next mixed with 1.0 ml of Greiss reagent (naphthylethylene diamine and sulfanilamide in phosphoric acid [14]). The nitrite standard curve (0, 1, 2, 3, 4, 6, 8, 10, and 20 μM concentrations) for each subject was generated in his/her own pooled plasma and the results were read at 540 nm on a spectrophotometer (DU-640; Beckman Instruments, Inc., Fullerton, CA). The sensitivity of this assay was determined to be 1.0 μM total nitrite. All data reported reflect total plasma nitrate and nitrite concentrations but are referred to as nitrite concentrations.

Calculations of forearm phenylalanine kinetics

The net forearm balances for glucose, lactate, oxygen, nitrite, and phenylalanine were calculated from the Fick principle:

$$\text{Net balance} = ([A] - [V]) \times F; \quad (1)$$

where [A] and [V] are arterial and venous substrate concentrations nd F is forearm blood flow in ml/min/100 ml forearm volume. As regards phenylalanine, the measurement of the absolute rates of syn-

1. *Abbreviations used in this paper:* L-NMMA, N^G-monomethyl-L-arginine; NNA, N^G-nitro-L-arginine; Phe, phenylalanine.

thesis and breakdown of muscle protein requires knowing the phenylalanine specific activity in the phenylalanyl-tRNA pool being used for protein synthesis. This is not experimentally accessible in the forearm. The rates of protein synthesis and degradation can be estimated from the kinetics of exchange of labeled phenylalanine across the forearm as described previously (6). Equations have been presented for estimating forearm protein synthesis and degradation that were based upon the use of either arterial or deep venous specific activity of [^{3}H]phenylalanine to approximate phenylalanyl-tRNA specific activity (16, 17). As phenylalanine is not synthesized, catabolized, or concentrated in muscle, the amount of phenylalanine extracted from arterial plasma (R_d) should at steady state reflect utilization of phenylalanine for muscle protein synthesis. R_d is given by the equation:

$$R_d = ([DPM_{art} - DPM_{vein}] \times flow)/SA_{art}. \quad (2)$$

R_a is a measure of tissue protein breakdown and is calculated from:

$$\text{Net balance} = R_d - R_a. \quad (3)$$

Data are presented using arterial specific activity to reflect the amino acid precursor pool as this is the manner in which these were reported previously (6).

Data presentation and statistical analysis

All data are presented as mean±SEM. Data on hormone concentrations, bilateral forearm substrate balance, and bilateral forearm amino acid kinetics are presented for each of the sample periods. For both protocols, samples are acquired at baseline, and after 4 (4 h) and 6 h (6 h) of the respective infusions. For protocol 1, data points are designated: basal (BAS), IGF alone (IGF), and IGF+L-NMMA (combined infusion). For protocol 2, data points are designated: basal (BAS), IGF+L-NMMA (4 h), and IGF+L-NMMA (6 h).

Within forearms, statistical comparisons were made using ANOVA for repeated measures and post-hoc, two-tailed comparisons with Duncan's test (True Epistat, Epistat Services, Richardson, TX).

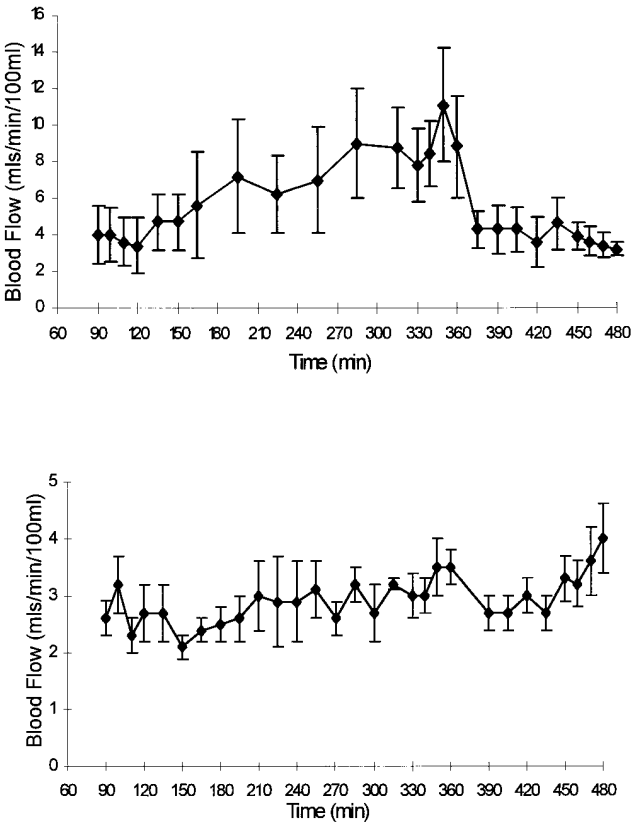

Figure 2. Blood flow responses. The top depicts the blood flow response in protocol 1, the bottom depicts protocol 2. Note the differences in the *y*-axis scales. Each data point reflects the mean±SEM value for that time point. Basal period = 90–120 min; 4 h = 330–360 min; 6 h = 450–480 min.

Results

General. All subjects tolerated the study without adverse reactions. Blood pressure did not change significantly in protocol 1 (basal: 137±6/56±6 mmHg; 4 h: 140±6/58±7 mmHg; 6 h: 144±6/54±6 mmHg at 6 h). In protocol 2, systolic (but not diastolic) blood pressure rose slightly, and significantly over the study (basal: 146±7/65±5 mmHg; 4 h: 156±8/63±5 mmHg; 6 h: 161±8/64±6 mmHg; ANOVA (systolic BP): $P < 0.005$; basal vs. 4 h or 6 h: $P < 0.05$).

Forearm blood flow. Fig. 2 (*top*) illustrates the changes in forearm blood flow over the course of the study. In protocol 1, forearm blood flow increased from the baseline mean of 3.8±1.0 to 7.8±1.9 ml/min/100 ml at 4 h (330–360 min) ($P < 0.01$). As seen in Fig. 2, blood flow declined rapidly in response to L-NMMA. During the 6-h (450–480 min) sample period, mean blood flow closely approximated the baseline value at 3.7±0.5 ml/min/100 ml (ANOVA: $P < 0.005$; 4 h vs. 6 h: $P < 0.01$). In protocol 2 (Fig. 2, *bottom*), the mean baseline flow was 2.7±0.3 and increased by 19% to 3.2±0.3 at 4 h and by an additional 9% to 3.5±0.5 ml/min/100 ml at 6 h. The overall increase in flow was restricted to 0.8±0.4 ml/min/100 ml or 28% over baseline (NS).

Substrate and hormonal concentrations. Table I summarizes the arterial and venous glucose and lactate concentrations and venous hormone concentrations for both protocols. Arterial glucose declined slightly but significantly in both protocols during the progressive fast. Insulin levels also declined during the study. Deep venous total IGF-I levels rose to levels observed previously (6) in both the IGF-infused as well as the contralateral arms. Table II provides arterial and venous phenylalanine concentrations and specific activities.

Metabolic responses to IGF-I and L-NMMA: glucose and lactate balances. Fig. 3 summarizes the changes in forearm glucose and lactate balance for protocol 1. At baseline, forearm glucose uptake was 0.5±0.1 μmol/min/100 ml, and in response to IGF-I it rose by 240% to 1.7±0.5 μmol/min/100 ml. With the infusion of L-NMMA and reduction in blood flow back to baseline, forearm glucose uptake remained elevated at 2.9±0.6 μmol/min/100 ml (ANOVA: $P < 0.005$; BAS vs. 4 h or 6 h: $P < 0.01$; 4 h vs. 6 h: NS). Similarly, forearm lactate release increased slightly, but insignificantly, between the basal and 4-h (IGF alone) periods, from 0.1±0.2 to −0.3±0.2 μmol/min/100 ml. By the 6-h time point, the increase in lactate release was significant at −0.6±0.1 μmol/min/100 ml (ANOVA: $P < 0.04$; BAS vs. 6 h: $P < 0.05$).

As observed in the 2-h L-NMMA study, the restraint of blood flow by L-NMMA in protocol 2 did not affect IGF-I's ability to stimulate forearm glucose uptake (Fig. 4). Despite the lack of increase in blood flow, forearm glucose uptake rose from 0.9±0.2 μmol/min 100 ml at baseline to 2.9±0.6 (4 h) and 2.9±0.8 (6 h) μmol glucose/min/100 ml (ANOVA: $P < 0.005$; BAS vs. 4 h or 6 h; $P < 0.01$). In parallel to the increase in glucose uptake, IGF+L-NMMA coinfusion briskly increased forearm lactate release, from 2.1±0.1 (BAS) to −0.8±0.2 (4 h)

Table I. Arterial and Venous Glucose and Lactate Concentrations and Hormone Concentrations, Protocols 1 and 2

	Protocol 1: IGF/IGF+L-NMMA				Protocol 2: IGF+L-NMMA			
	BAS	4 h	6 h	*P*	BAS	4 h	6 h	*P*
Glu Art	4.5±0.1	4.3±0.2	4.2±0.2*	< 0.05	4.6±0.2	4.5±0.1	4.3±0.2	< 0.05
Glu Vein	4.4±0.1	4.0±0.2‡	3.5±0.2‡	< 0.001	4.3±0.2	3.4±0.2	3.4±0.2	< 0.001
Glu CL Vein	4.3±0.1	4.2±0.2	4.0±0.2‡	< 0.01	4.3±0.2	4.2±0.2	4.2±0.2	NS
Lac Art	0.8±0.2	0.6±0.1	0.6±0.1	NS	0.7±0.1	0.5±0.1	0.5±0.1	NS
Lac Vein	0.8±0.2	0.7±0.1	0.7±0.1	NS	0.7±0.1	0.8±0.1	0.8±0.1	NS
Lac CL Vein	0.8±0.1	0.7±0.1	0.7±0.1	NS	0.7±0.2	1.1±0.6	0.5±0.1	NS
Insulin	22±8	9±4§	5±2§	< 0.001	44±5	18±6‡	13±6‡	< 0.001
IGF Vein	167±23	604±32‡	849±21‡‖	< 0.0001	225±19	822±70§	871±61§	< 0.0001
IGF CL Vein	176±20	345±34‡	404±33‡‖	< 0.001	213±17	400±32‡	465±33‡‖	< 0.001

Data are expressed as mean±SEM. Glucose (*Glu*) and lactate (*Lac*) concentrations in μmol/liter. Insulin concentrations in pmol/liter. IGF-I concentrations in ng/ml. *CL*, contralateral vein indicates the control or noninfused arm. *P* indicates ANOVA by repeated measures within arm. Post-hoc testing by Duncan's test: ‡$P < 0.001$ vs. BAS; §$P < 0.01$ vs. BAS; ‖$P < 0.05$ vs. 4 h; *$P < 0.05$ vs. BAS. Vein refers to the IGF and L-NMMA infused arm.

and −0.9±0.2 (6 h) μmol/min/100 ml (ANOVA: $P < 0.005$; BAS vs. 4 h or 6 h: $P < 0.01$).

Forearm phenylalanine kinetics. Figs. 5 and 6 depict the changes in phenylalanine (Phe) balance, R_a, and R_d in both protocols. In protocol 1, the infusion of IGF-I alone induced a significant, positive, and anabolic shift in phenylalanine balance, from −22±2 to +17±7 nmol/min/100 ml between the basal and 4-h sample points (Fig. 5, *top*). Restriction of flow to baseline did not affect this value at 6 h (+22±7 nmol/min/100 ml). This change in phenylalanine balance was statistically significant (ANOVA: $P < 0.0001$; BAS vs. 4 h or 6 h: $P < 0.001$) and was due to both a 46% decrease in Phe R_a (ANOVA: $P < 0.03$; BAS vs. 6 h: $P < 0.05$; Fig. 5, *middle*) and a 50% increase in R_d (ANOVA; $P < 0.02$; BAS vs. 4 h or 6 h: $P < 0.05$; Fig. 5, *bottom*).

In protocol 2, the simultaneous infusion of IGF+L-NMMA did not alter the positive change in phenylalanine balance (−21±3 to 14±4 nmol Phe/min/100 ml, ANOVA: $P < 0.0001$; BAS vs. 6 h: $P < 0.001$, Fig. 6, *top*). Like the effect of IGF-I alone or in combination with L-NMMA, in protocol 1 Phe R_a fell by 46% (Fig. 6, *middle)* (ANOVA: $P < 0.005$; BAS vs. 6 h: $P < 0.005$). In contrast to the effect observed in protocol 1, there was a slight, but insignificant, 25% rise in Phe R_d at 6 h (Fig. 6, *bottom*).

Forearm oxygen uptake. In protocol 1, mean baseline forearm oxygen uptake was 8.5±1.3 μmol/min/100 ml. After 4 h of IGF-I O_2 uptake rose to 10.7±2.2 μmol/min/100 ml, and by 6 h to 13.8±2.2 μmol/min/100 ml during the combined infusion (ANOVA: $P < 0.01$; BAS vs. 6 h: $P < 0.01$). In protocol 2, forearm oxygen uptake rose in the IGF+L-NMMA arm from 8.5±0.8 to 9.8±0.8 (4 h) to 12.1±1.3 (6 h) μmol/min/100 ml (ANOVA: $P < 0.04$; BAS vs. 6 h: $P < 0.03$).

Forearm nitrite balance. Table III summarizes the arterial and venous concentrations as well as forearm balances of nitrite in protocol 1. The values reported include plasma nitrate and nitrite. Mean basal arterial nitrite levels declined significantly by 4 h ($P < 0.01$) and further by 6 h (ANOVA: $P < 0.0001$; BAS vs. 6 h: $P < 0.001$; 4 h vs. 6 h: $P < 0.05$). Between basal and 6 h, venous nitrite concentrations in both the IGF-I–infused and the contralateral arms also fell across the study, paralleling the change in the arterial nitrite levels. Despite the decline in arterial nitrite concentrations, forearm nitrite release increased markedly between the basal and IGF alone period ($P < 0.05$). L-NMMA returned forearm nitrite balance back to baseline, a change that was also significantly different from the 4 h (IGF alone) value (ANOVA: $P < 0.03$; 4 h vs. 6 h: $P < 0.05$). No changes in nitrite release were observed in the contralateral arm during the course of the study.

Table II. Phenylalanine Concentrations and Specific Activities in Protocols 1 and 2

	Protocol 1: IGF/IGF+L-NMMA				Protocol 2: IGF+L-NMMA			
	BAS	4 h	6 h	*P*	BAS	4 h	6 h	*P*
Phe Art	47±3	43±6*	43±4*	< 0.01	49±2	46±2	46±2	NS
Phe Vein	53±3	41±2‡	37±3‡	< 0.001	56±3	44±3	42±4	< 0.001
Phe CL Vein	54±3	51±3	48±3‡	< 0.001	58±2	54±2	54±3	NS
Phe SA_a	18±2	21±2	22±2*	< 0.05	20±2	21±2	21±2	NS
Phe SA_v	13±1	18±2‡	16±2‡	< 0.001	14±1	17±2‡	16±1‡	< 0.001
Phe $SACL_v$	12±2	12±2	15±2*	< 0.05	13±1	15±2*	15±1*	< 0.01

Data are expressed as mean±SEM. Phenylalanine (*Phe*) concentrations in μmol/liter. Phenylalanine specific activities (*SA*) in dpm/nmol to nearest integer. *CL*, contralateral. *P* indicates ANOVA by repeated measures within arm. Post-hoc testing by Duncan's test: ‡$P < 0.01$ vs. BAS; *$P < 0.05$ vs. BAS.

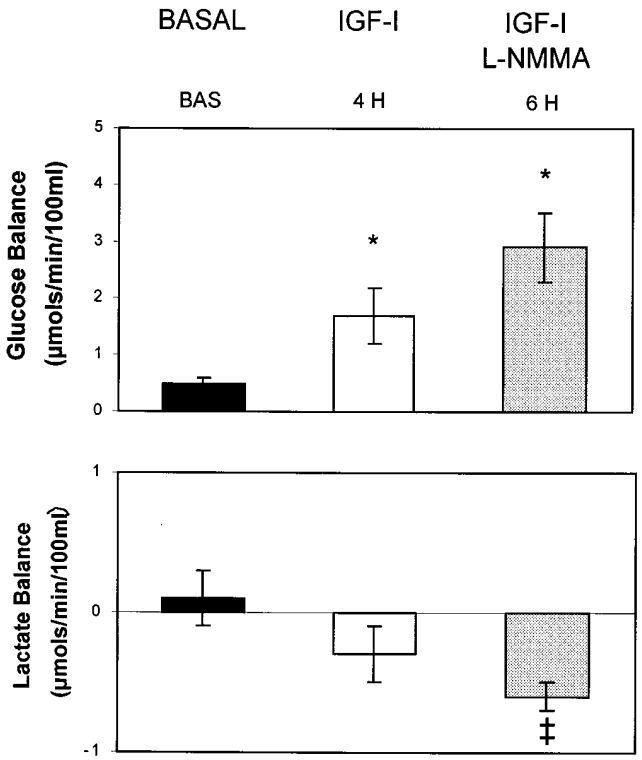

Figure 3. Forearm glucose and lactate responses, protocol 1. The top depicts forearm glucose balance and the bottom depicts forearm lactate balance during this protocol. $^{*}P < 0.01$ vs. BAS. $^{\ddagger}P < 0.05$ vs. BAS.

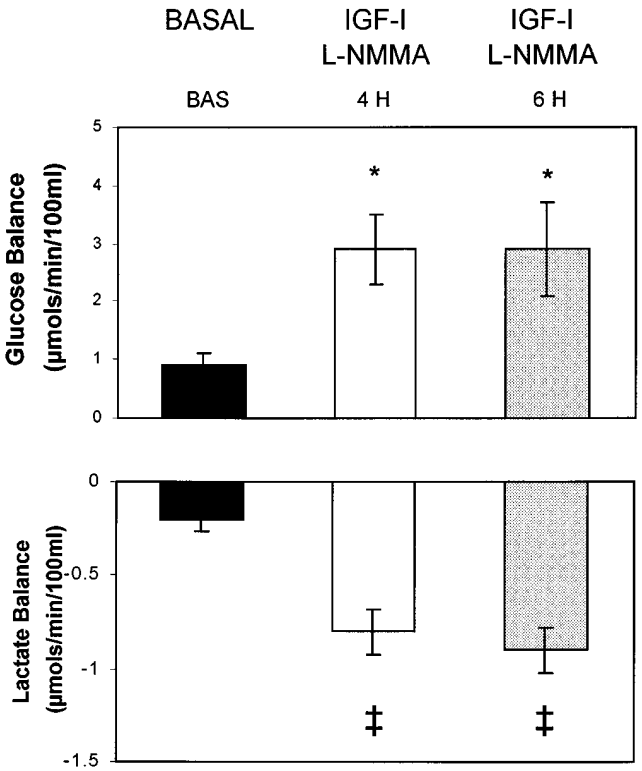

Figure 4. Forearm glucose and lactate responses, protocol 2. The top depicts forearm glucose balance and the bottom depicts forearm lactate balance during this protocol. $^{*}P < 0.01$ vs. BAS; $^{\ddagger}P < 0.02$ vs. BAS.

Contralateral arm. Table IV summarizes the changes in forearm blood flow, glucose, lactate, oxygen, and phenylalanine balances, as well as phenylalanine, R_a and R_d for both protocols 1 and 2. The rise in contralateral forearm flow in protocol 1 was not statistically significant and largely due to a substantial rise in a single subject. In protocol 2, the increase in flow was statistically significant. Despite time-dependent elevations in blood flow in the control arms, forearm oxygen, lactate, and phenylalanine balances and phenylalanine kinetics were stable across the study during both protocols. In protocol 1, contralateral forearm glucose uptake decreased transiently at 4 h and returned to baseline by 6 h.

Discussion

Three major observations were made from these studies. First, the increase in blood flow and nitrite due to IGF-I was inhibitable by a competitive antagonist of nitric oxide synthase, suggesting that IGF-I mediates the increase in blood flow by augmenting nitric oxide production. Second, despite normalization of flow by L-NMMA during the last 2 h of protocol 1, IGF-I exerted both its insulin- and growth hormone–like actions. Third, in contrast to observations made in protocol 1, the titration or clamping of blood flow at the basal level for the entire experiment (protocol 2) antagonized the IGF-stimulated increase in muscle protein synthesis.

In the last several years, it has been demonstrated that several different vasodilators, including acetylcholine, bradykinin, and histamine, exert their effects on vascular smooth muscle through endothelial cell generation of nitric oxide (18–20). As observed for these vasodilators, in vitro, IGF-I stimulates the production of nitric oxide in both human umbilical vein cells and immortalized rat renal artery cells (10) and, in vivo, in both humans and animals, systemically infused IGF-I increases renal blood flow (21–23), a response which can be blocked in rats by the administration of L-NMMA before IGF-I infusion (24).

The results of this study extend these observations and provide two pieces of in vivo evidence that the IGF-stimulated increase in forearm blood flow is nitric oxide dependent. First, L-NMMA rapidly restored to normal the IGF-mediated increase in blood flow or, when infused continuously in protocol 2, completely blocked its increase. These observations are similar to results from other experiments using L-NMMA in the forearm, i.e., vasodilation due to a nitric oxide–dependent vasodilator is antagonized by nitric oxide synthase inhibition (8, 9, 25–28).

The second piece of evidence that IGF-I increases blood flow through nitric oxide is that IGF-I increases nitrate and nitrite production, an effect that is entirely abolished by L-NMMA. Several in vivo studies have demonstrated previously that serum nitrate/nitrite levels rise in response to sepsis (29–32), gastroenteritis (33), heart failure (34), during the follicular phase of the menstrual cycle (35), and in response to an infusion of L-arginine (36). Some of these studies have also reported that the increase in nitrate and nitrite production can be blunted by nitric oxide synthase inhibition (31, 32). That forearm nitrate/nitrite release increases due to IGF-I in this study is concordant with these other observations but is distinguished from them by the increase in nitrate/nitrite production detected from within the forearm.

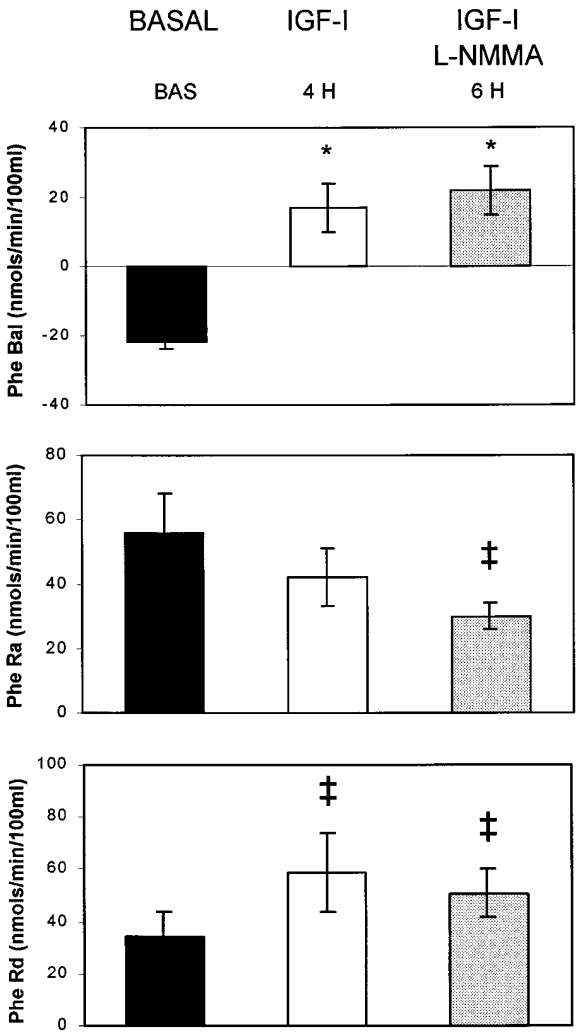

Figure 5. Forearm phenylalanine balance, R_a, and R_d, protocol 1. The top, middle, and bottom panels, respectively, depict forearm phenylalanine balance, R_a, and R_d. $^*P < 0.001$ vs. BAS; $^‡P < 0.05$ vs. BAS.

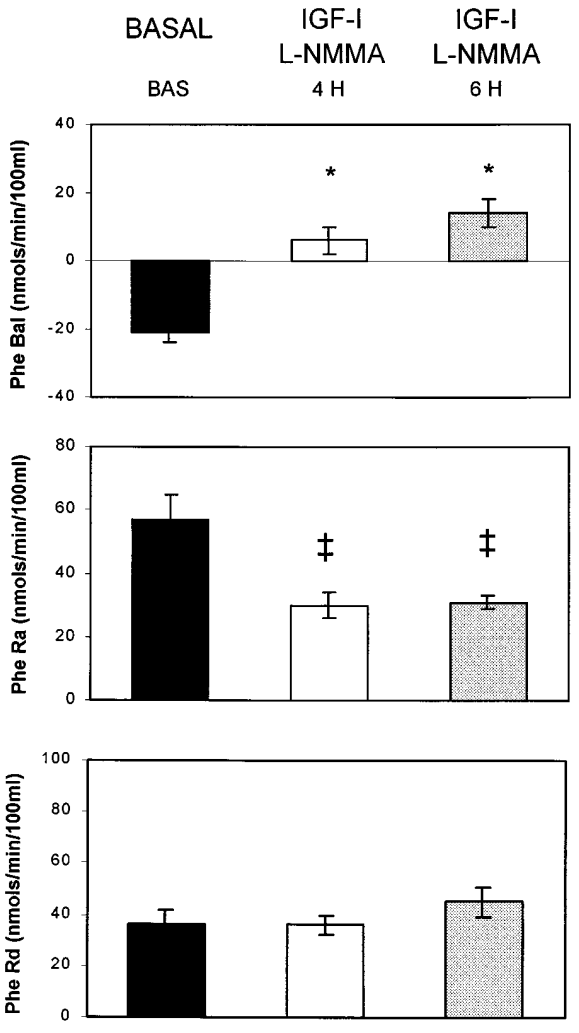

Figure 6. Forearm phenylalanine balance, R_a, and R_d, protocol 2. The top, middle, and bottom panels, respectively, depict forearm phenylalanine balance, R_a, and R_d. $^*P < 0.001$ vs. BAS; $^‡P < 0.005$ vs. BAS.

In addition to significant changes in local production, the decline in arterial (as well as venous) nitrate/nitrite concentrations was striking. At baseline, the mean arterial and venous levels were lower than many already published values (29, 30, 34, 35). However, it should be pointed out that it is not reported in most studies whether or not the subjects were fasting and at bed rest before the samples were drawn (29, 30, 34, 35). In two studies, in which these conditions were defined, values are closer to or below those reported in the present study (36, 37). This issue of fasting and bed rest also likely explains the decline in plasma nitrate/nitrite levels over time. At present, no other time course data are available to which these results can be compared. However, based upon urinary nitrate/nitrite excretion in mice and humans the most likely explanation for this decline is progressive fasting (38, 39). Although it has been demonstrated clearly that nitrate and nitrite are synthesized in mammalian cells, increasing dietary nitrate intake similarly increases urinary excretion of nitrate/nitrite in both mice (39) and humans (38). Placing the subject on a nitrate-restricted diet decreases, but does not eliminate, urinary nitrate production (38). The subjects in this study begin fasting at 8 p.m. the evening before the study and continue to fast until the end of the study at approximately 4 p.m. the next day. By the basal sample period, which is usually drawn at 10 a.m., each subject will have fasted for at least 13–14 h. Thus, one possibility is that there is continued renal clearance of plasma nitrate/nitrite during the fast as well as diminished intestinal contributions from food and/or bacteria. In addition, as exercise increases nitric oxide production (27, 40, 41), it needs to be considered that activity before blood sampling may also alter plasma nitrate/nitrite concentrations.

Although the concept that IGF-I has potent vasodilatory effects differs from a more conventional view of growth factors primarily causing cellular differentiation and tissue growth, precedent for the vasoactive roles of growth factors has been observed recently with insulin (8, 9, 42, 43). Several investigators have reported increases in limb (either forearm or leg) blood flow during euglycemic hyperinsulinemia (8, 9, 42, 43). The magnitude of the insulin-induced increase in blood flow was $\sim$ 75–100% over the baseline value and correlated well with whole body glucose uptake in both lean and obese subjects (3, 4). This effect of insulin on blood flow is dose dependent in lean subjects (3, 4) and appears to be blunted in obese or hypertensive insulin-resistant patients (3, 5). As a result of

Table III. Arterial and Venous Nitrite Concentrations and Forearm Nitrite Balances, Protocol 1

	BAS	IGF alone	IGF+L-NMMA	*P*
Nitrite artery (μM)	10.4±3.5	7.2±2.6*	5.4±1.9‡§	< 0.0001
Nitrite vein (μM)	10.6±3.7	8.0±3.1*	5.2±2.3‡§	< 0.0001
Nitrite contralateral vein (μM)	10.6±3.8	7.4±2.7*	5.4±2.7‡§	< 0.0005
IGF arm nitrite balance	−0.4±0.9	−7.7±2.8‖	0.4±1.3§	< 0.03
Contralateral nitrite balance	−0.2±0.3	−0.7±0.6	0.2±1.0	NS

Data are expressed as mean±SEM. Nitrite balance data are in units of nmol nitrite/min/100 ml forearm volume. *P* indicates ANOVA by repeated measures within arm. Vein refers to the IGF and L-NMMA infused arm. Contralateral vein indicates the control or noninfused arm. Post-hoc testing by Duncan's test: ‡$P < 0.001$ vs. BAS; *$P < 0.01$ vs. BAS; §$P < 0.05$ vs. 4 h; ‖$P < 0.05$ vs. BAS.

these compelling observations, it has been hypothesized that the augmentation of blood flow by insulin contributes to or modulates glucose disposal in skeletal muscle (44).

Scherrer and colleagues recently presented evidence that nitric oxide mediates the vasodilation due to insulin (8). In this study, healthy, lean subjects were given a step-wise, 16-min infusion of L-NMMA into the brachial artery before a 2-h, systemic infusion of insulin (8). Prior treatment with L-NMMA blocked the insulin-induced, ~ 40% rise in blood flow that was observed in the control study. The authors noted that whole body glucose disposal was not altered by intraarterial L-NMMA; forearm glucose uptake values were not measured (8). Similarly, Baron et al. (9) infused insulin systemically for ~ 220 min using two different doses of insulin. In both the low and high doses, which yielded insulin levels of 69 and 210 μU/ml respectively, leg blood flow rose similarly and to approximately twice that of baseline (9). After the insulin infusion period, L-NMMA was infused directly into the femoral artery for 1 h at 16 mg/min. At this dose of L-NMMA, leg glucose uptake fell from 107 to 80 mg/min with the low dose and 114 to 85 mg/min with high-dose insulin. The authors concluded that insulin's effects on blood flow contributed 25% of insulin's overall effect to stimulate glucose uptake (9). The present study demonstrates that IGF-induced increase in glucose uptake (as well as the insulin-like action on proteolysis) is not at all dependent upon an increase in blood flow.

In contrast to systemic insulin administration, insulin infusion directly into the brachial artery does not increase blood flow markedly (15). Only at a dose yielding deep venous insulin concentrations of 125 μU/ml did insulin augment blood flow and, in that experiment, by 25% (15). At lower doses yielding deep venous insulin levels comparable with those observed in systemic insulin infusion studies, no increases in forearm blood flow were observed (45). This contrast suggests that the mechanism for insulin augmentation of blood flow may be outside of the limb, e.g., activation of the sympathetic nervous system (43, 46, 47). It is important to point out that this increase in limb blood flow due to either systemically or locally infused insulin has not been uniformly observed (48, 49). However, as insulin and IGF-I are similar in both structure and effects and that L-NMMA blocks the insulin-mediated increase in flow, the results of the present study are compared with those studies that have observed an increase in blood flow due to insulin.

Unlike insulin, IGF-I markedly increases forearm blood flow when infused directly into the brachial artery (6, 7). At the lowest dose tested, which does not significantly raise forearm glucose uptake, IGF-I increases forearm blood flow and the Phe R_d by 75 and 69%, respectively (6). At the same dose used in the present study, in a previous study IGF-I alone doubled blood flow by 4 h, tripled flow by 6 h, and exerted significant metabolic effects. These actions include a four- to sevenfold increase in glucose uptake and a marked, anabolic shift in forearm phenylalanine balance due to a large increase in Phe R_d (synthesis) as well as a decrease in Phe R_a (proteolysis). It is important to point out that this dose of IGF-I yields an equivalent effect on forearm glucose uptake as the dose of insulin that increases forearm flow by 25% (15). Within the forearm preparation and when normalized for effects on glucose uptake, therefore, IGF-I has a greater effect on blood flow than does insulin.

The association between IGF-I's action on blood flow and its metabolic effects prompted the present study. It is not possible to sort out from this earlier work whether or not the increase in flow was required to observe these effects. This requirement may be due to a simple mathematical effect, as blood flow is a multiplier in every equation used to calculate

Table IV. Metabolic Responses in Contralateral Arms of Protocols 1 and 2

	Protocol 1: IGF/IGF+L-NMMA				Protocol 2: IGF+L-NMMA			
	BAS	4 h	6 h	*P*	BAS	4 h	6 h	*P*
Blood flow	3.4±1.0	3.9±1.3	5.1±1.6	NS	2.3±0.3	3.4±0.4	4.3±0.9*	< 0.02
Glucose Bal	0.7±0.2	0.3±0.1‡	0.5±0.2	< 0.001	0.7±0.2	1.1±0.6	0.5±0.1	NS
Lactate Bal	0.0±0.3	−0.2±0.1	−0.4±0.1	NS	−0.3±0.1	−0.4±0.1	−0.6±0.1	NS
Oxygen Bal	10.1±1.3	8.8±1.5	9.7±1.7	NS	9.8±1.3	8.9±0.9	10.3±1.8	NS
Phe Bal	−21±1	−21±5	−22±6	NS	−20±1	−25±3	−31±8	NS
Phe R_a	62±10	64±10	65±17	NS	47±4	52±6	61±11	NS
Phe R_d	41±10	43±12	42±14	NS	27±3	27±5	30±5	NS

Data are expressed as mean±SEM. Blood flow in units of ml/min/100 ml forearm volume. Glucose, lactate, and oxygen balances (*Bal*) in μmol/min/100 ml. Phenylalanine balances (*Phe Bal*), R_a, and R_d in nmol/min/100 ml. *P* indicates ANOVA by repeated measures within arm. Post-hoc testing by Duncan's test: ‡$P < 0.01$ vs. BAS; *$P < 0.05$ vs. BAS.

net substrate balance as well as phenylalanine kinetics. Part of this concern is alleviated by examination of the direction of changes for these outcome variables. Qualitative shifts from net release to net uptake (or a change in the arterio-venous difference from negative to positive, as observed for phenylalanine) provide some assurance that the observed outcome is likely independent of the numeric change in flow.

Addressing whether or not the increase in flow, per se, was responsible for the observed outcome is more difficult. That the increase in flow, in general, becomes manifest before the metabolic change suggests that the two may be causally related. However, examination of a variety of circumstances under which forearm blood flow increases aids with this distinction. For example, when epinenephrine is infused into the brachial artery at a dose that raises deep venous epinephrine levels to those observed in severe stress, blood flow approximately doubles and glucose uptake falls (50). However, muscle protein anabolism is achieved through an insulin-like mechanism, i.e., decrease in proteolysis. Chloroquine, the antimalarial thought to inhibit lysosome activity, doubles blood flow in the forearm yet does not affect forearm glucose and phenylalanine handling (51). Finally, intraarterially or systemically administered growth hormone increases flow by approximately twofold and increases Phe R_d by $\sim$ 70% (13). Despite these marked increases in flow, the metabolic outcomes induced by these agents are quite distinct from each other. As regards glucose uptake, none of the above increase glucose extraction, in fact, both growth hormone and epinephrine antagonize insulin's action (52). Thus, analysis of forearm tissue metabolic responses in the context of increases in flow suggests that flow responds to differing metabolic demands in muscle.

If flow does not directly affect muscle metabolism, it is still possible that the increase in flow facilitates these metabolic responses by augmenting perfusion of tissue that would not have been exposed to as much substrate, hormone, etc. In contrast to epinephrine, growth hormone, or chloroquine, the change in total flow or distribution may be more critical for hormones such as insulin and IGF-I, which promote glucose uptake. That restoration of flow to baseline for 2 h or clamping flow at baseline for 6 h did not alter glucose uptake, lactate release, or suppression of proteolysis supports the alternative hypothesis, namely that the muscle metabolic response to IGF-I is intrinsic to muscle and not dependent upon the flow response. That is, IGF will increase the uptake of a total mass of glucose (or phenylalanine or oxygen) either by increasing blood flow or by increasing the arterio-venous extraction ratio for that substrate. Therefore, net balance is the key outcome variable and, in response to IGF-I, muscle adjusts fractional substrate extraction to yield the desired shift in total substrate mass.

These data demonstrate that the insulin-like actions of IGF-I are not dependent upon an increase in flow. The responses observed in protocol 1 demonstrate that acute reduction in flow by L-NMMA does not alter the forearm muscle metabolic response. In addition to the insulin-like responses to IGF-I, a similar conclusion was reached for the growth hormone–like effects, i.e., Phe R_d rose during the increase in forearm blood flow and when forearm blood flow was returned to baseline. This latter observation was particularly critical as increases in Phe R_d (protein synthesis) have been accompanied consistently by increases in flow (6, 13). As flow is a multiplier in the calculation of Phe R_d (see Methods), the persistent elevation of Phe R_d despite the reduction in flow by L-NMMA in protocol 1 suggests that flow did not mathematically alter the Phe R_d at the 6-h time point.

However, the aforementioned discussion focuses on total forearm blood flow. It is not possible at present to distinguish alterations in the distribution of flow within a tissue bed. As muscle is not uniform in fiber type and hence metabolic activity, it has been proposed that alterations in the distribution of blood flow within muscle might alter its overall metabolic activity (2, 53). That is, some areas may be more metabolically active than others and in response to a metabolic stimulus, there is increased perfusion of more metabolically active areas (termed nutritive flow [53]). Not all metabolic stimuli may yield the same pattern of nutritive versus nonnutritive flow, such that similar increases in total blood flow may not be equal. As this concept relates to this study and other studies with L-NMMA, it needs to be considered that although total blood flow was restored to baseline, the relative distribution of nutritive to nonnutritive flow may not be the same. This issue will require further study.

Despite the lack of an acute effect of flow reduction on these outcome variables, it was possible that an initial increase in flow (and nitric oxide synthase activity) was sufficient to set these metabolic events in motion. To address this possibility, in protocol 2 L-NMMA was infused to prevent any significant increase in blood flow throughout the entire 6-h period. Clamping blood flow to basal values throughout the entire study did not change the insulin-like effects of IGF-I, i.e., there were substantive rises in glucose uptake and lactate release as well as an anabolic shift in phenylalanine balance due to suppression of muscle proteolysis. The only metabolic outcome that is distinct from protocol 1 is that no significant increase in Phe R_d was observed. It is important to emphasize that this increase in Phe R_d has been readily detected with IGF-I alone (Fryburg, D., both published and unpublished data) or in protocol 1 in combination with a 2-h infusion of L-NMMA. Therefore, it is unlikely that this attenuated response reflects a false negative result. In addition, it is possible that L-NMMA partially blocked protein synthesis in forearm muscle not by antagonizing nitric oxide synthase, but rather as substrate for protein synthesis. As aminoacyl-tRNA synthetases can discriminate minor differences in amino acid structure with a high degree of specificity, it is unlikely that L-NMMA directly antagonizes protein synthesis. Recent studies from this laboratory with L-NMMA alone (at the same doses used in this study) do not affect muscle protein synthesis or, for that matter, glucose balance (Fryburg, D., unpublished results).

That L-NMMA antagonizes IGF-mediated increase in protein synthesis is consistent with the observation that hepatic protein synthesis in septic rats is blocked by N^G-nitro-L-arginine (NNA), another inhibitor of nitric oxide synthase (32). In that study, rats injected with endotoxin responded with large increases in plasma nitrate/nitrite concentrations and increases in fractional hepatic protein synthesis. Another group of animals received NNA in a single dose before administration of endotoxin. Pretreatment with NNA blocked the increase in nitrate/nitrite at both 4 and 10 h after endotoxin and attenuated the rise in hepatic protein synthesis at 4 but not 10 h after endotoxin (32). In contrast, nitric oxide generated in vitro from Kupffer cells in response to lipopolysaccharide inhibited cultured hepatocyte protein synthesis (54). However, in rat mesangial cells laminin synthesis increased twofold and collagen and fibronectin synthesis decreased by 35–48% in response to

either γ-interferon or lipopolysaccharide (55). N^G-nitro-L-arginine (L-NAME), another inhibitor of nitric oxide synthase, blocked these changes in protein synthesis (55). Taken together, nitric oxide appears to be involved in the modulation of protein synthesis in a variety of tissues, responses which may depend upon the tissue and the stimulus for nitric oxide generation.

In addition to its physiologic significance, these observations have import for diabetes mellitus. Several groups have demonstrated that the vasomotor responses to either nitric oxide production or inhibition are impaired in patients or animals with diabetes mellitus (56–60). This effect appears to be more manifest in patients with microalbuminuria than those with normal urinary albumin excretion (60). This impairment in nitric oxide–dependent vasodilation is consistent with the blunted vasodilatory response to insulin-resistant patients (3, 4). It is presently unknown if the blood flow response to IGF-I is also impaired in patients with diabetes mellitus.

Up to this point, the discussion has centered upon blood flow and therefore the endothelial cell isoform of nitric oxide synthase. In addition to endothelial cell nitric oxide synthase, two other major isoforms have been identified, i.e., neuronal (brain) and inducible (61). Of particular relevance to the present study, the neuronal form appears to be highly expressed in human skeletal muscle (62). The abundance of nitric oxide synthase in skeletal muscle might suggest a potential metabolic role of nitric oxide in this tissue. Recently, two different groups of investigators have demonstrated that, in vitro, muscle contraction stimulates rat skeletal muscle production of nitric oxide (63, 64). Kobzik and colleagues also observed that nitric oxide synthase blockade augments, and nitric oxide donors depress, muscle contractility (64). Balon and Nadler observed that blockade of nitric oxide synthase in vitro decreases exercise-induced glucose uptake (63). Thus, early observations suggest that nitric oxide is important in skeletal muscle metabolism. In addition to affecting endothelial cell nitric oxide synthase, it also needs to be considered that IGF-I also affects skeletal muscle nitric oxide synthase and that the nitrate/nitrite measured in the present study may have originated in skeletal muscle as well as endothelium. Furthermore, it is acknowledged that the infusion of L-NMMA (or other nitric oxide synthase inhibitors in this and any other study) may also inhibit the skeletal muscle isoform in addition to the endothelial isoform. Thus, the diminished effect on muscle protein synthesis may be a reflection of the inhibition of muscle and not endothelial nitric oxide synthase.

In conclusion, this study demonstrated that IGF-I increases blood flow through a nitric oxide–dependent mechanism and that the increase in flow is not required to observe the metabolic actions of IGF-I alone. Therefore, an increase in flow is not critical to the expression of IGF-I's actions in normal young adults. However, the attenuation of the effect on muscle protein synthesis raises the possibility that IGF-I's effect on protein synthesis may depend upon nitric oxide production in muscle. These results suggest the need for further studies examining the regulation of nitric oxide synthase in skeletal muscle.

Acknowledgments

The author thanks Linda Jahn and the nursing staff of the University of Virginia General Clinical Research Center for their excellent conduct of the study and care of the subjects, Diana Oliveras and Rachel Walsh for excellent technical assistance, and Dr. Eugene J. Barrett and Dr. Stephen Rattigan for the constructive suggestions regarding this study and the manuscript. Human recombinant IGF-I was provided by Genentech, Inc.

This work was supported by U.S. Public Health Service grants AR01881, DK38578, and RR00847 to the University of Virginia General Clinical Research Center.

References

1. Hudlicka, O. 1985. Regulation of muscle blood flow. *Clin. Physiol. (Oxf.).* 5:201–209.

2. Duling, B.R., and D.H. Damon. 1985. An examination of the measurement of flow heterogeneity in striated muscle. *Circ. Res.* 60:1–13.

3. Laakso, M., S.V. Edelman, G. Brechtel, and A.D. Baron. 1990. Decreased effect of insulin to stimulate skeletal muscle blood flow in obese man. A novel mechanism for insulin resistance. *J. Clin. Invest.* 85:1844–1852.

4. Baron, A.D., and G. Brechtel. 1993. Insulin differentially regulates systemic and skeletal muscle vascular resistance. *Am. J. Physiol.* 265:E61–E67.

5. Baron, A.D., G. Brechtel-Hook, A. Johnson, and D. Hardin. 1993. Skeletal muscle blood flow: a possible link between insulin resistance and blood pressure. *Hypertension (Dallas).* 21:129–135.

6. Fryburg, D.A. 1994. Insulin-like growth factor I exerts growth hormone- and insulin-like actions on human muscle protein metabolism. *Am. J. Physiol.* 267:E331–E336.

7. Copeland, K.C., and K.S. Nair. 1994. Recombinant human insulin-like growth factor-I increases forearm blood flow. *J. Clin. Endocrinol. & Metab.* 79: 230–232.

8. Scherrer, U., D. Randin, P. Vollenweider, L. Vollenweider, and P. Nicod. 1994. Nitric oxide release accounts for insulin's vascular effects in humans. *J. Clin. Invest.* 94:2511–2515.

9. Baron, A.D., H.O. Steinberg, H. Chaker, R. Leaming, A. Johnson, and G. Brechtel. 1995. Insulin-mediated skeletal muscle vasodilation contributes to both insulin sensitivity and responsiveness in lean humans. *J. Clin. Invest.* 96: 786–792.

10. Tsukahara, H., D.V. Gordienko, B. Tonshoff, M.C. Gelato, and M.S. Goligorsky. 1994. Direct demonstration of insulin-like growth factor-I-induced nitric oxide production by endothelial cells. *Kidney Int.* 45:598–604.

11. Sigdell, Jan-E. 1975. Venous occlusion plethysmography. Part 1: Basic principles and applications. Biomed. Eng. (Berl.). 10:300–349.

12. Jorfeldt, L.S. 1988. Measurement of skeletal muscle blood flow in humans: plethysmographic, bolus and continuous infusion technique. *Am. J. Cardiol.* 62:E25–E29.

13. Fryburg, D.A., R.A. Gelfand, and E.J. Barrett. 1991. Growth hormone acutely stimulates muscle protein synthesis in normal humans. *Am. J. Physiol.* 260:E499–E504.

14. Tracey, W.R. 1992. Spectrophotometric detection of nitrogen oxides using azo dyes. *Neuroprotocols.* 1:125–131.

15. Gilliam, M.B., M.P. Sherman, J.M. Griscavage, and L.J. Ignarro. 1993. A spectrophotometric assay for nitrate using NADPH oxidation by Aspergillus nitrate reductase. *Anal. Biochem.* 212:359–365.

16. Gelfand, R.A., and E.J. Barrett. 1987. Effect of physiologic hyperinsulinemia on skeletal muscle protein synthesis and breakdown in man. *J. Clin. Invest.* 80:1–6.

17. McNulty, P.H., L.H. Young, and E.J. Barrett. 1993. Response of rat heart and skeletal muscle protein in vivo to insulin and amino acid infusion. *Am. J. Physiol.* 264:E958–E966.

18. Ignarro, L.J. 1988. Biological actions and properties of endothelium-derived nitric oxide formed and released from artery and vein. *Circ. Res.* 65:1–21.

19. Moncada, S., and A. Higgs. 1993. The L-arginine-nitric oxide pathway. *N. Engl. J. Med.* 329:2002–2012.

20. Billiar, T.R. 1995. Nitric oxide: novel biology with clinical relevance. *Ann. Surg.* 221:339–349.

21. Hirschberg, R., and J.D. Kopple. 1989. Evidence that insulin-like growth factor I increases renal plasma flow and glomerular filtration rate in fasted rats. *J. Clin. Invest.* 83:326–330.

22. Hirschberg, R., and J.D. Kopple. 1989. Effects of growth hormone and IGF-I on renal function. *Kidney. Int.* 36:S20–S26.

23. Jaffa, A.A., D. LeRoith, C.T. Roberts, Jr., P.F. Rust, and R.K. Mayfield. 1994. Insulin-like growth factor I produces renal hyperfiltration by a kinin-mediated mechanism. *Am. J. Physiol.* 266:F102–F107.

24. Haylor, J., I. Singh, and A.M. El Nahas. 1991. Nitric oxide synthesis inhibitor prevents vasodilation by insulin-like growth factor I. *Kidney Int.* 39:333–335.

25. Vallance, P., J. Collier, and S. Moncada. 1989. Effects of endothelium-derived nitric oxide on peripheral arteriolar tone in man. *Lancet.* ii:997–1000.

26. Lefroy, D.C., T. Crake, N.G. Uren, G.J. Davies, and A. Maseri. 1993. Effect of inhibition of nitric oxide synthesis on epicardial coronary artery cali-

ber and coronary blood flow in humans. *Circulation.* 88:43–54.

27. Endo, T., T. Imaizumi, T. Tagawa, M. Shiramoto, S. Ando, and A. Takeshita. 1994. Role of nitric oxide in exercise-induced vasodilation of the forearm. *Circulation.* 90:2886–2890.

28. McKie, L.D., B.L. Bass, B.J. Dunkin, and J.W. Harmon. 1994. Nitric oxide mediates the blood flow response to intravenous adenosine in the rabbit. *Circ. Shock*. 43:103–106.

29. Wong, H.R., J.A. Carcillo, G. Burckart, N. Shah, and J.E. Janosky. 1995. Increased serum nitrite and nitrate concentrations in children with the sepsis syndrome. *Crit. Care Med.* 23:835–842.

30. Ochoa, J.B., A.O. Udekwu, T.R. Billiar, R.D. Curran, F.B. Cerra, R.L. Simmons, and A.B. Pietzman. 1991. Nitrogen oxide levels in patients after trauma and during sepsis. *Ann. Surg.* 214:621–626.

31. Tracey, W.R., J. Tse, and G. Carter. 1995. Lipopolysaccharide-induced changes in plasma nitrate and nitrate concentrations in rats and mice: pharmacological evaluation of nitric oxide synthase inhibitors. *J. Pharmacol. Exp. Ther.* 272:1011–1015.

32. Frederick, J.A., P.O. Hasselgren, S. Davis, T. Higashiguchi, T.D. Jacob, and J.E. Fisher. 1993. Nitric oxide may upregulate in vivo hepatic protein synthesis during endotoxemia. *Arch. Surg.* 128:152–157.

33. Jungersten, L., A. Edlund, L.O. Hafstrom, L. Karlsson, A.-S. Petersson, and A. Wennmalm. 1993. Plasma nitrate as an index of immune system activation in animals and man. *J. Clin. Lab. Immunol.* 40:1–4.

34. Winlaw, D.S., G.A. Smythe, A.M. Keogh, C.G. Schyvens, P.M. Spratt, and P.S. Macdonald. 1994. Increased nitric oxide production in heart failure. *Lancet*. 344:373–374.

35. Rosselli, M., B Imthurm, E. Macas, P.J. Keller, and R.K. Dubey. 1994. Circulating nitrite/nitrate levels increase with follicular development: indirect evidence for estradiol mediated release. *Biochem. Biophys. Res. Commun.* 202: 1543–1552.

36. Kharitonov, S.A., G. Lubec, B. Lubec, M. Hjelm, and P.J. Barnes. 1994. L-Arginine increases exhaled nitric oxide in normal human subjects. *Clin. Sci.* 88:135–139.

37. Guarner, C., G. Soriano, A. Tomas, O. Bulbena, M.T. Novella, J. Balanzo, F. Vilardell, M. Mourelle, and S. Moncada. 1993. Increased serum nitrite and nitrate levels in patients with cirrhosis: relationship to endotoxemia. *Hepatology.* 18:1139–1143.

38. Green, L.C., K. Ruiz de Luzuriaga, D.A. Wagner, W. Rand, N. Istfan, V.R. Young, and S.R. Tannenbaum. 1981. Nitrate biosynthesis in man. *Proc. Natl. Acad. Sci. USA*. 78:7764–7768.

39. Granger, D.L., J.B. Hibbs, Jr., and L.M. Broadnax. 1991. Urinary nitrate excretion in relation to murine macrophage activation. *J. Immunol.* 146:1294–1302.

40. Sun, D., A. Huang, A. Koller, and G. Kaley. 1994. Short-term daily exercise activity enhances endothelial NO synthesis in skeletal muscle arterioles of rats. *J. Appl. Physiol.* 76:2241–2247.

41. Matsumoto, A., Y. Hirata, S. Momomura, H. Fujita, A. Yao, M. Sata, and T. Serizawa. 1994. Increased nitric oxide production during exercise. *Lancet* 343:849–850.

42. Creager, M.A., C.S. Liang, and J.D. Coffman. 1985. Beta adrenergic-mediated vasodilator response to insulin in the human forearm. *J. Pharmacol. Exp. Therap.* 235:709–714.

43. Anderson, E.A., R.P. Hoffman, T.W. Balon, C.A. Sinkey, and A.L. Mark. 1991. Hyperinsulinemia produces both symptomatic neural activation and vasodilation in normal humans. *J. Clin. Invest.* 87:2246–2252.

44. Baron, A.D. 1994. Hemodynamic actions of insulin. *Am. J. Physiol.* 267: E187–E202.

45. Louard, R.J., D.A. Fryburg, R.A. Gelfand, and E.J. Barrett. 1992. Insulin sensitivity of protein and glucose metabolism in human forearm skeletal muscle. *J. Clin. Invest.* 90:2348–2354.

46. Rowe, J.W., J.B. Young, K.L. Minaker, A.L. Stevens, J. Pallotta, and L. Landsberg. 1981. Effect of insulin and glucose infusions on sympathetic nervous system activity in normal man. *Diabetes*. 30:219–225.

47. Vollenweider, P., L. Tappy, D. Randin, P. Schneiter, E. Jequier, P. Nicod, and U. Scherrer. 1993. Differential effects of hyperinsulinemia and carbohydrate metabolism on sympathetic nerve activity and muscle blood flow in humans. *J. Clin. Invest.* 92:147–154.

48. Natali, A., G. Buzzigoli, S. Taddei, D. Santoro, M. Cerri, R. Pedrinelli, and E. Ferrannini. 1990. Effects of insulin on hemodynamics and metabolism in human forearm. *Diabetes.* 39:490–500.

49. Yki-Jarvinen, H., A.A. Young, C. Lamkin, and J.E. Foley. 1987. Kinetics of glucose disposal in whole body and across the forearm in man. *J. Clin. Invest.* 79:1713–1719.

50. Fryburg, D.A., R.A. Gelfand, L.A. Jahn, D.M. Oliveras, R.S. Sherwin, L. Sacca, and E.J. Barrett. 1995. Effects of epinephrine on human muscle glucose and protein metabolism. *Am. J. Physiol.* 268:E55–E59.

51. Barrett, E.J., L.A. Jahn, D.M. Oliveras, and D.A. Fryburg. 1995. Chloroquine does not exert insulin-like actions on human forearm muscle metabolism. *Am. J. Physiol.* 268:E820–E824.

52. Fryburg, D.A., R.J. Louard, K.E. Gerow, R.A. Gelfand, and E.J. Barrett. 1992. Growth hormone stimulates skeletal muscle protein synthesis and antagonizes insulin's antiproteolytic action in humans. *Diabetes*. 41:424–429.

53. Clark, M.G., E.Q. Colquhoun, S. Rattigan, K.A. Dora, T.P. Eldershaw, J.L. Hall, and J. Ye. 1995. Vascular and endocrine control of muscle metabolism. *Am. J. Physiol.* 268:E797–E812.

54. Billiar, T.R., R.D. Curran, D.J. Stuehr, F.K. Ferrari, and R.L. Simmons. 1989. Evidence that activation of Kupffer cells results in production of L-arginine metabolites that release cell-associated iron and inhibit hepatocyte protein synthesis. *Surgery (St. Louis).* 106:364–372.

55. Trachtman, H., S. Futterweit, and P. Singhal. 1985. Nitric oxide modulates the synthesis of extracellular matrix proteins in cultured rat mesangial cells. *Biochem. Biophys. Res. Commun.* 207:120–125.

56. Mayhan, W.G. 1989. Impairment of endothelium-dependent dilatation of cerebral arterioles during diabetes mellitus. *Am. J. Physiol.* 256:H621–H625.

57. McVeigh, G.E., G.M. Brennan, G.D. Johnston, B.J. McDermott, L.T. McGrath, W.R. Henry, J.W. Andrews, and J.R. Hayes. 1992. Impaired endothelium-dependent and independent vasodilation in patients with type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia*. 35:771–776.

58. Calver, A., J. Collier, and P. Vallance. 1992. Inhibition and stimulation of nitric oxide synthesis in the human forearm arterial bed of patients with insulin-dependent diabetes. *J. Clin. Invest.* 90:2548–2554.

59. Johnstone, M.T., S.J. Creager, K.M. Scales, J.A. Cusco, B.K. Lee, and M.A. Creager. 1993. Impaired endothelium-dependent vasodilation in patients with insulin-dependent diabetes mellitus. *Circulation.* 88:2510–2516.

60. Elliott, T.G., J.R Cockcroft, P.-H. Groop, G.C. Viberti, and J.M. Ritter. 1993. Inhibition of nitric oxide synthesis in forearm vasculature of insulin-dependent diabetes patients: blunted vasoconstriction in patients with microalbuminuria. *Clin. Sci.* 85:687–693.

61. Forstermann, U., H.H.H.W. Schmidt, J.S. Pollock, H. Scheng, J.A. Mitchell, T.D. Warner, M. Nakane, and F. Murad. 1991. Isoforms of nitric oxide synthase. *Biochem. Pharmacol.* 42:1849–1857.

62. Nakane, M., H.H.H.W. Schmidt, J.S. Pollock, U. Föstermann, and F. Murad. 1992. Cloned human brain nitric oxide synthase is highly expressed in skeletal muscle. *FEBS Lett.* 316:175–180.

63. Balon, T.W., and J.L. Nadler. 1994. Nitric oxide release is present from incubated skeletal muscle preparations. *J. Appl. Physiol.* 77:2519–2521.

64. Kobzik, L., M.B. Reid, D.S. Bredt, and J.S. Stamler. 1994. Nitric oxide in skeletal muscle. *Nature (Lond.).* 372:546–548.