Physical Inactivity Rapidly Induces Insulin Resistance and Microvascular Dysfunction in Healthy Volunteers

Naomi M. Hamburg, **Craig J. McMackin**, **Alex L. Huang**, **Sherene M. Shenouda**, **Michael E. Widlansky**, **Eberhard Schulz**, **Noyan Gokce**, **Neil B. Ruderman**, **John F. Keaney Jr**, and **Joseph A. Vita**
From the Evans Department of Medicine and Whitaker Cardiovascular Institute, Boston University School of Medicine, Boston, Mass

Abstract

Objectives—Sedentary lifestyle increases the risk of cardiovascular disease and diabetes. Vascular dysfunction contributes to atherogenesis and has been linked to insulin resistance.

Methods and Results—We measured insulin sensitivity by glucose tolerance test and vascular function by ultrasound and venous occlusion plethysmography in 20 healthy subjects (14 men, 6 women) at baseline and during 5 days of bed rest. Bed rest led to a 67% increase in the insulin response to glucose loading (*P*<0.001) suggesting increased insulin resistance and produced increases in total cholesterol and triglycerides. Bed rest led to decreased reactive hyperemia in the forearm (1317±404 to 1112±260 mL/min, *P*=0.01) and the calf (28.5±7.0 to 22.2±8.7 mL/min/dL, *P*=0.003) indicating impaired microvascular function. Bed rest decreased brachial artery diameter and increased systolic blood pressure suggesting increased basal arterial tone. There were no changes in circulating inflammatory markers arguing against systemic inflammation as a mechanism for vascular dysfunction in this setting.

Conclusions—Physical inactivity was associated with the development of insulin resistance, dyslipidemia, increased blood pressure, and impaired microvascular function in healthy volunteers. Our findings may provide insight into the pathogenesis of vascular disease in sedentary individuals and emphasize that even short-term physical inactivity may have adverse metabolic and vascular consequences.

Keywords

insulin resistance; sedentary lifestyle; obesity; endothelium; blood pressure; reactive hyperemia

Sedentary lifestyle increases the risk of developing cardiovascular disease and diabetes.[1,2] Public health investigations indicate that more than 200 000 cardiovascular deaths per year can be attributed to physical inactivity in the United States.[3] Despite compelling evidence that physical inactivity is detrimental to cardiovascular health, over one quarter of all Americans engage in no leisure time physical activity.[4] The pathways leading from a sedentary lifestyle to insulin resistance and atherosclerosis are incompletely understood.

Dysfunction of the vascular endothelium contributes to atherogenesis[5] and has been linked to sedentary lifestyle. In cross-sectional studies, sedentary individuals have impaired endothelial

vasomotor function compared with those who are physically active.[6] Sedentary individuals also display impaired reactive hyperemia,[7] which is the increase in blood flow that occurs after transient ischemia and is a complex response that reflects both endothelium-dependent and endothelium-independent dilation of resistance vessels.[8] States of insulin resistance, including type 2 diabetes mellitus and obesity, are also associated with endothelial dysfunction.[9]

Previous studies have demonstrated that short periods of inactivity lead to insulin resistance in humans.[10–12] We hypothesized that insulin resistance induced by short-term physical inactivity would be associated with vascular dysfunction. This finding would lend further support to basic studies suggesting that insulin resistance and vascular dysfunction share common mechanisms.[13] Thus, we assessed vascular function and glucose tolerance before and after a 5-day period of strict bed rest in healthy subjects.

Materials and Methods

Subjects

Healthy nonsmoking volunteers were recruited for this study by newspaper and internet advertisement. Subjects were eligible if they had no clinical history of hypertension, diabetes mellitus, or hyperlipidemia, and were not taking any prescription medications. We sought individuals with preserved flow-dependent vasodilation at baseline, and therefore, excluded subjects if brachial artery flow-mediated dilation was less than 8% at a screening visit. This cut point was selected because it identified a low risk subgroup in a prior prospective study from our laboratory.[14] The Institutional Review Board of Boston Medical Center approved the protocol and all subjects provided written informed consent.

Study Protocols

Subjects completed a screening visit to determine eligibility. Up to a month later, they were admitted to the Boston Medical Center General Clinical Research Center for the 5-day study protocol. Subjects were asked to maintain their usual activity level before the study admission. For women, the testing period was scheduled to occur within the follicular period based on previous studies demonstrating stability of vascular function in this phase of the menstrual cycle.[15]

All subjects began the protocol on a Monday. They arrived in the morning after fasting overnight and had assessment of vascular function and insulin sensitivity, as described below. On completion of baseline studies, subjects began the bed rest period. Subjects remained in bed and were permitted to perform quiet activities such as reading, watching television, and talking on the telephone. Subjects were allotted up to 30 minutes out of bed over the course of each 24-hour period. This time was restricted to activities necessary to perform personal hygiene. A stopwatch was used to monitor all time spent out of bed. The nursing staff monitored subjects to insure adherence to the activity requirements. A clinical nutritionist performed a 24-hour food recall on admission and provided food to match their usual dietary habits. On the third study day (Wednesday), vascular function testing was repeated, and on the fifth study day (Friday), vascular function and assessment of insulin sensitivity were repeated at the same time of day as the baseline evaluation.

As a time and activity control, we assessed vascular function and indices of insulin sensitivity in 9 additional subjects. After baseline testing of vascular function and glucose tolerance, these subjects were instructed to perform their usual level of physical activity. Repeat testing was then performed under the same conditions 5 days later.

Measurement of Vascular Function

Vascular function testing was completed in the morning as previously described.[16,17] Endothelium-dependent flow-mediated dilation of the conduit brachial artery was assessed by comparing arterial diameter in 2-dimensional images at baseline and 1 minute after a 5-minute cuff occlusion of the upper arm. After a 10-minute rebaseline period, we assessed endothelium-independent dilation of the same arterial segment before and 4 minutes after sublingual nitroglycerin (0.4 mg). The nitroglycerin portion was omitted if systolic blood pressure was <100 mm Hg or if the patient had prior migraine headaches or adverse reaction to nitroglycerin. Flow-mediated dilation was expressed as the absolute change in diameter in millimeters and as the percent increase in diameter from baseline.

Reactive hyperemia is emerging as a important measure of microvascular function that depends in part on endothelium-derived nitric oxide, is blunted in the setting of cardiovascular risk factors, and relates to cardiovascular events.[18–20] We assessed peak reactive hyperemia using Doppler ultrasound to record flow velocity in the brachial artery before and after 5-minute upper arm cuff occlusion. We measured average flow velocity during 5 cardiac cycles immediately after cuff release (peak flow), as previously described.[16] Images were digitized, and arterial diameters and Doppler flows were measured with customized software (Medical Imaging Applications, Inc) by individuals blinded to clinical and laboratory status of the subjects.

To gain additional insight into bed rest-induced changes in vascular function, we examined leg blood flow responses using venous occlusion plethysmography in a subset of 10 subjects. Our subjects were allowed to use their arms during the bed rest protocol; thus, vascular function in the lower extremities may have been particularly affected. We also wanted to examine the entire hyperemic response, because the later stages may be more nitric oxide–dependent.[21] The leg blood flow measurements were made using a mercury-in-silastic strain gauge placed on the calf, upper thigh and ankle cuffs, and a computerized plethysmograph (Hokanson Inc). During measurements, circulation to the foot was excluded by inflating the ankle cuff to supra-systolic pressure. Five measurements of basal flow were acquired with thigh cuff inflation to 40 mm Hg. After 5 minutes of thigh cuff inflation to supra-systolic pressures, blood flow recordings were made every 15 seconds for a total of 1.5 minutes.

Measurement of Glucose Tolerance

After the vascular function testing, we performed an oral glucose tolerance test.[22] Briefly, we measured fasting glucose and insulin levels at baseline and 30, 60, 90, and 120 minutes after the subjects drank a solution containing 75 g of glucose (Trutol, NERL Diagnostics Inc). Net glucose and insulin responses to glucose loading were calculated as the area under the curve over 2 hours. Insulin sensitivity was assessed by 2 validated measures. (1) Homeostasis model assessment-insulin resistance (HOMA-IR) was calculated using the following formula: HOMA-IR=[(fasting insulin μIU/mL)×(fasting glucose mmol/L)]/22.5[23] and (2) $ISI_{0,120}$ was calculated as (m/MPG)/log MSI, m is [75,000 mg+(fasting glucose-2-hour glucose) ×0.19×body wt (kg)]/120 minutes, MPG is the mean of fasting and 2-hour glucose concentrations (mg/dL), and MSI is the mean of fasting and 2-hour insulin concentrations (mU/L).[24]

Biochemical Analyses

Total cholesterol, HDL cholesterol, triglycerides, and glucose were measured using enzymatic methods. LDL cholesterol was calculated using the Friedewald formula.[25] Insulin was measured using immunochemiluminometric methodology (Quest Diagnostics). Serum C-reactive protein was measured using a high-sensitivity nephelometric method as previously

described.[26] Serum interleukin (IL)-6, tumor necrosis factor (TNF) receptor-II, and adiponectin were measured using commercially available kits (R&D Systems Inc).

Statistical Analysis

Values are reported as mean±SD unless otherwise specified. Total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, weight, and glucose were measured on Days 1 and 5 and compared using the paired *t* test. The Wilcoxon signed rank test was used to compare insulin, HOMA-IR, and C-reactive protein on Days 1 and 5, because these variables did not have a normal distribution based on the Shapiro-Wilk test. Systolic blood pressure, diastolic blood pressure, heart rate, flow-mediated dilation, nitroglycerin mediated dilation, baseline brachial artery diameter, baseline brachial flow, baseline flow velocity, hyperemic flow, hyperemic flow velocity, IL-6, TNF receptor-II, and adiponectin were measured on Days 1, 3, and 5 and compared using repeated-measures analysis of variance (RM-ANOVA). We used 2-way RM-ANOVA to compare the insulin and glucose responses to the oral glucose load as well as the calf hyperemic responses on Days 1 and 5. Statistical significance was accepted at a probability value of <0.05. With the sample size of 20 subjects, the current study had 80% power (alpha=0.05) to detect a change of 22% (1.6 percentage points) in flow-mediated dilation and 15% (170 mL/min) in hyperemic flow, which are comparable to the differences between sedentary and active individuals.[27]

Results

Baseline Characteristics

Twenty-two healthy subjects, including 14 men and 8 women (mean age, 30.7±8 years) participated in the bed rest protocol. Two subjects (both female) withdrew before completing the protocol. Nine control subjects participated in the normal activity protocol (5 men, 4 women, mean age, 28.8±6.6 years). At baseline, serum cholesterol, insulin, glucose, and systolic and diastolic blood pressure were within normal limits in all subjects.

Physical Inactivity and Glucose Tolerance and Insulin Sensitivity

As shown in Table 1, bed rest increased fasting glucose levels, insulin levels, and HOMA-IR, and decreased $ISI_{0,120}$. Figure 1 shows the time course of insulin and glucose response to an oral glucose load. The net insulin response(area under the curve) increased by 67% after bed rest compared with baseline (*P*<0.001 by RM-ANOVA). There was also a 6% increase in the net glucose response (*P*=0.03 by RM-ANOVA).

Physical Inactivity and Microvascular Vasodilator Function

Figure 2 displays the effect of bed rest on peak hyperemia flow in the arm measured by Doppler ultrasound. As shown, peak hyperemic response was lower on Day 3 (1,078±268 mL/min, *P*=0.009 versus baseline) and Day 5 (1113±261 mL/min, *P*=0.02 versus baseline) of bed rest as compared with the baseline active state (1317±404 mL/min, *P*=0.01 for overall RM-ANOVA).

Figure 3 displays the effect of bed rest on the total hyperemic response in the leg measured by venous occlusion plethysmography. As shown, there was a 29% decrease in total hyperemic response of the leg (*P*<0.05 by RM-ANOVA). Compared with the active state, peak hyperemic response in the leg also decreased significantly after bed rest (28.5±7.0 mL/min/dL versus 22.2±8.7 mL/min/dL, *P*=0.003).

There were no significant correlations between the bed rest-induced change in reactive hyperemic in the arm and leg and changes in HOMA-IR, $ISI_{0,120}$, or the insulin response to glucose loading.

As shown in Table 2, there was no significant change in either baseline flow or baseline flow velocity in the brachial artery during bed rest. Baseline flow decreased in the leg after bed rest as measured by plethysmography (2.4±0.9 mL/min/dL versus 3.1±1.2 mL/min/dL in the active state, *P*=0.02).

Physical Inactivity and Conduit Vessel Vasodilator Function

As shown in Table 2, baseline brachial artery diameter decreased during bed rest as compared with the active state. There was no effect of bed rest on flow-mediated dilation measured as the absolute change or the percent change. Because flow-mediated dilation remained unchanged despite the reduction in hyperemic flow (the stimulus for dilation), we examined the effect of bed rest on the ratio of these 2 variables. As shown in Table 2, this ratio increased significantly during bed rest. Although there also was a tendency for increased nitroglycerin-mediated dilation during bed rest in the subset of patients with available data, this change was not statistically significant, likely reflecting the smaller sample size.

Physical Inactivity and Blood Markers and Hemodynamics

Bed rest was associated with significant increases in total cholesterol and triglycerides (Table 1) and systolic blood pressure (Table 2). There was also a trend toward higher diastolic blood pressure and LDL cholesterol after bed rest. There was no effect of bed rest on body weight, heart rate, or HDL cholesterol. There was no correlation between the bed rest–induced changes in triglyceride levels and markers of insulin resistance including HOMA and the net insulin response (area under the curve).

As shown in Table 3, there was no effect of bed rest on systemic markers of inflammation, including C-reactive protein, IL-6, and TNF receptor-II, and there was no effect on adiponectin levels. There were no significant correlations between the bed rest–induced change in reactive hyperemic and changes in lipids, blood pressure, or markers of inflammation (data not shown).

Normal Activity and Measures of Vascular Function and Insulin Sensitivity

In the 9 control subjects, there were no changes after 5 days of normal activity in fasting glucose, fasting insulin, insulin AUC, glucose AUC, or lipid parameters (data not shown). Similarly there were no changes in hyperemic flow in the forearm (1005±138 mL/min versus 952±160 mL/min, p=NS) or in the leg (Figure 3). Baseline arterial diameter, baseline flow, flow-mediated dilation, and blood pressure remained unchanged with usual activity (data not shown).

Discussion

This study demonstrated a significant reduction in reactive hyperemia in the upper and lower extremities of healthy volunteers after a short period of bed rest. This impairment of microvascular function was accompanied by a marked increase in the insulin response to an oral glucose load and by increased fasting insulin, glucose levels, and HOMA-IR, changes that are consistent with the development of insulin resistance. Flow-mediated dilation, which reflects vasodilator function of the conduit brachial artery, was not impaired during bed rest, suggesting a preferential impairment of microvascular function. Bed rest was also associated with a decrease in baseline arterial diameter and resting flow and an increase in systolic blood pressure, consistent with increased basal arterial tone and vascular resistance. Furthermore, we observed increases in total cholesterol and triglycerides after bed rest, consistent with the metabolic changes associated with insulin resistance. These changes occurred without changes in systemic inflammatory markers or adiponectin levels. There were no changes observed in vascular function or indices of insulin sensitivity in a control group examined before and after 5 days of usual activity. Collectively, these findings support the hypothesis that physical

inactivity concurrently induces a state of insulin resistance and vascular dysfunction in healthy volunteers.

Several lines of evidence support a link between physical inactivity and insulin resistance. Individuals with a sedentary lifestyle have increased risk of developing Type 2 diabetes mellitus.[1] Insulin resistance has been shown to develop with prolonged physical inactivity and after only 3 days of bed rest.[10] Our study design was based on these observations, and we sought to examine vascular function in a group of previously healthy individuals before and during the earliest stages of insulin resistance.

Several prior studies examined the effect of physical inactivity on microvascular function, but used much more prolonged or restrictive protocols than the present study. For example, decreased reactive hyperemia[28] and a decreased blood flow response to acetylcholine infusion[29] have been observed after 2 weeks of physical inactivity, but those studies used head-down bed rest, an intervention that produces marked volume shifts that may influence blood flow responses.[30] In the present study, vascular dysfunction developed after less than 48 hours, even though subjects were allowed to get out of bed for up to 30 minutes per day. Thus, prolonged periods or extreme forms of physical inactivity are not required, making our observations more relevant to hospitalized patients and sedentary individuals.

Previous studies also examined conduit artery function in the setting of physical inactivity. Consistent with the present study, a decrease in resting femoral artery diameter was observed after prolonged bed rest[31] or limb suspension.[32] Observational studies have demonstrated impaired conduit artery function in patients with chronic sedentary lifestyle[6] or insulin resistance.[13] Paradoxically, we observed an increase in flow-mediated dilation when adjusting for the change in reactive hyperemia, and prior investigators have reported similar results.[31,32] Because those prior studies also demonstrated an increase in nitroglycerin-mediated dilation, investigators have argued that these findings might reflect increased responsiveness of vascular smooth muscle.[31] However, interpretation of the conduit artery responses in this setting is complicated by the concurrent changes in arterial diameter and blood pressure. Smaller baseline arterial size or increased resting arterial tone might lead to proportionally higher flow-mediated dilation that does not reflect improved dilator function. Further studies will be required to determine the mechanisms that account for our observation that short-term bed rest apparently has directionally opposite effects on microvascular and conduit artery vasodilator function.

We considered the possibility that a systemic proinflammatory state might account for impaired microvascular function in our study, because sedentary lifestyle and states of insulin resistance are associated with altered levels of circulating cytokines and adipocytokines.[33–35] However, we observed no increase in serum levels of C-reactive protein, IL-6, or soluble TNF receptor II, and no decrease in adiponectin, a protective adipocyte-derived protein.[35] Although it remains possible that local inflammation at the level of vascular tissue might have played a role, these findings argue against a systemic state of inflammation or significant alterations in adipocytokine levels as mechanisms for the present findings.

The concurrent development of vascular dysfunction, insulin resistance, increased blood pressure, and dyslipidemia in response to bed rest suggests that these phenomena might share a common mechanism. In cross-sectional studies, states of insulin resistance have been associated with impaired endothelium-dependent vasodilation in peripheral microvessels.[36] In the setting of cardiovascular risk factors, including obesity and Type 2 diabetes mellitus, reactive hyperemia is blunted,[18,19] reflecting, in part, endothelial dysfunction.[18,21] Experimental studies have shown that insulin action and activation of endothelial nitric oxide synthase depend on common signaling mechanisms, including the phosphatidylinositol-3

kinase (PI3 kinase)/Akt system and AMP-dependent protein kinase,[13,37] and endothelial nitric oxide synthase null mice display insulin resistance,[38] providing further support for a direct link between insulin resistance and vascular dysfunction. Prior studies have also suggested that endothelial dysfunction might contribute to the development of insulin resistance as reduced blood flow led to decreased peripheral insulin-mediated glucose uptake.[39] Thus, abnormal glucose tolerance observed with bed rest may be related to reduced peripheral flow, which we observed in the lower extremities of the studied subjects.

A variety of other mechanisms might also contribute to the development of vascular dysfunction during bed rest. For example, the decrease in resting blood flow and the resulting reduction in local shear stress might decrease the expression of endothelial nitric oxide synthase.[40] Decreased flow might induce production of reactive oxygen species and endogenous vasoconstrictors that alter vascular function.[41] Further studies will be needed to characterize the precise mechanisms accounting for the rapid development of vascular dysfunction and insulin resistance during physical inactivity in humans.

Our study has a number of limitations. First, we cannot exclude the possibility that alterations in diet might have contributed to our findings, although the in-hospital diets were determined by a nutritionist's assessment. Second, use of a euglycemic clamp would have provided a more precise assessment of insulin sensitivity. Third, the study was limited to noninvasive assessment of vascular function, and our measure of microvascular function was reactive hyperemia. On the other hand, reactive hyperemia is fundamental response of the vasculature that determines tissue perfusion, is impaired with cardiovascular risk factors,[19] and predicts cardiovascular events.[20] As physical inactivity has multiple potential and observed physiological effects, our study design precludes the establishment of a definitive causal relationship between insulin resistance and vascular dysfunction. The participants were not randomized to normal activity or bed rest; thus, we cannot exclude the possibility that differences between the 2 groups influenced our findings. These limitations are balanced by the relatively large sample size and the marked changes in insulin sensitivity and vascular function in response to a clinically relevant form of physical inactivity.

In summary, we observed the concurrent development of insulin resistance, microvascular dysfunction, dyslipidemia, and increased blood pressure following a short period of bed rest in healthy volunteers. There is a growing epidemic of diseases associated with physical inactivity and insulin resistance including obesity, the metabolic syndrome, and diabetes mellitus.[42] Our data are consistent with the hypothesis that insulin resistance has deleterious effects on the vasculature or that common pathophysiological mechanisms account for both effects of bed rest. Our study suggests that the vasculature is highly sensitive to the metabolic changes associated with physical inactivity. On this basis, we speculate that even short periods of inactivity could adversely affect hospitalized patients, and conversely, a program of increased physical activity and exercise might have rapid benefits in previously sedentary patients.

Acknowledgements

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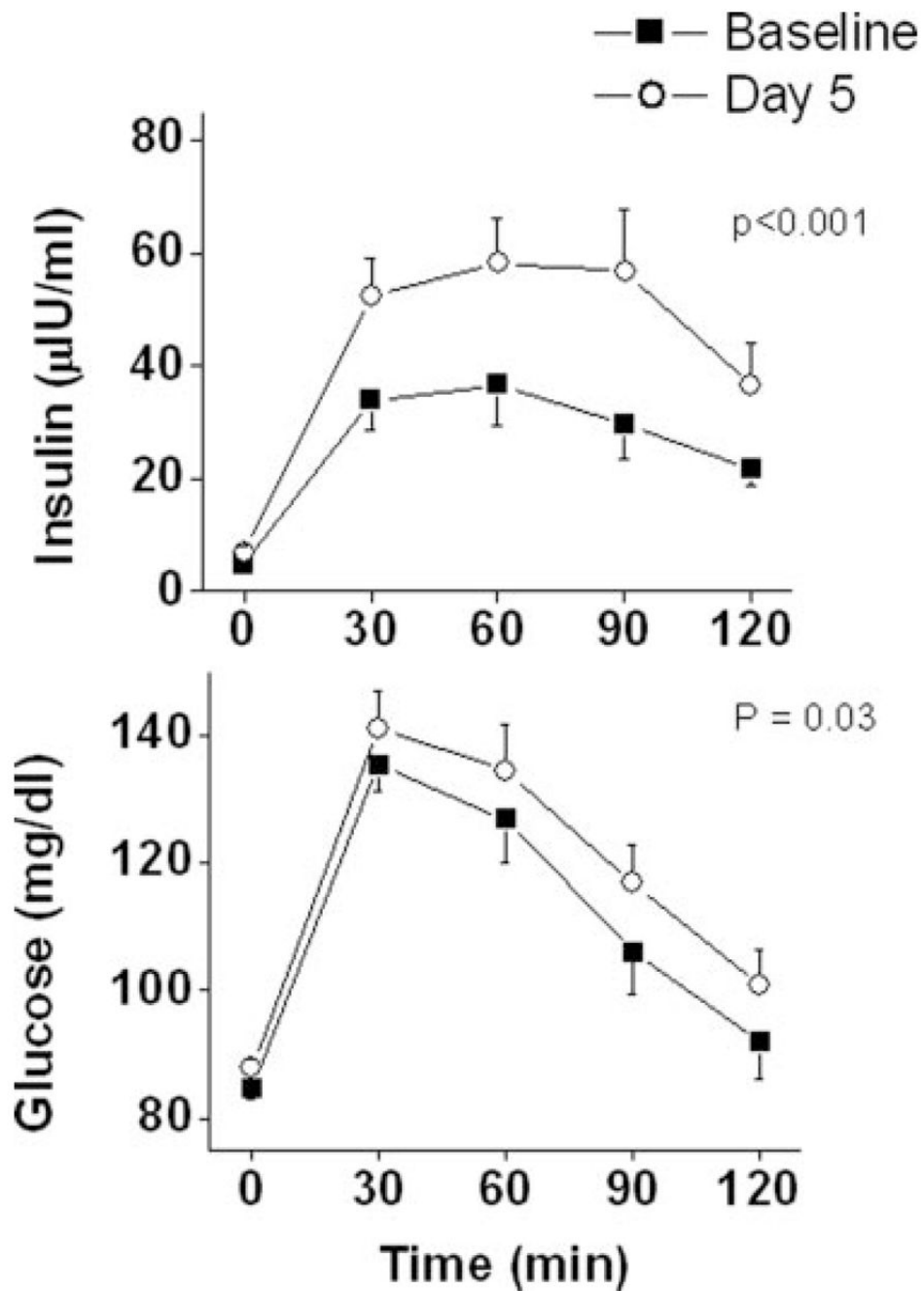
Figure 1.
The effect of bed rest on the insulin (top) and glucose (bottom) response to an oral glucose load in 20 healthy subjects. Bed rest was associated with an increase in the insulin response (*P*<0.001) and the glucose response (*P*=0.03), reflecting the development of insulin resistance. Data are mean±SE.
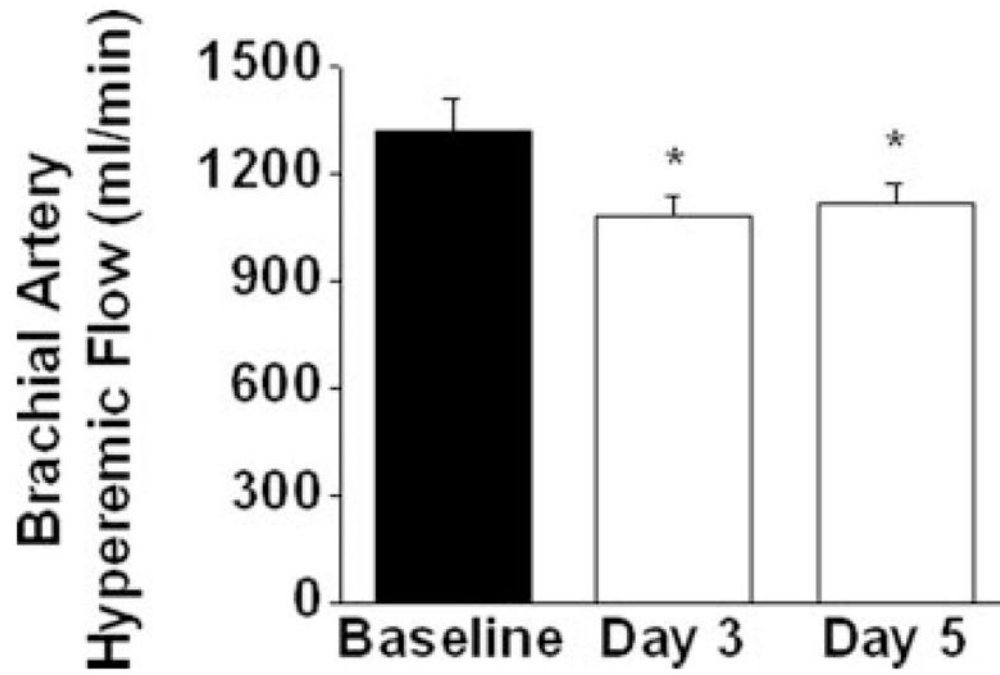
Figure 2.
Reactive hyperemia measured by Doppler ultrasound was blunted with bed rest in the upper extremity (*P*<0.01 for ANOVA, *P<0.05, n=19). Data are mean±SEM.
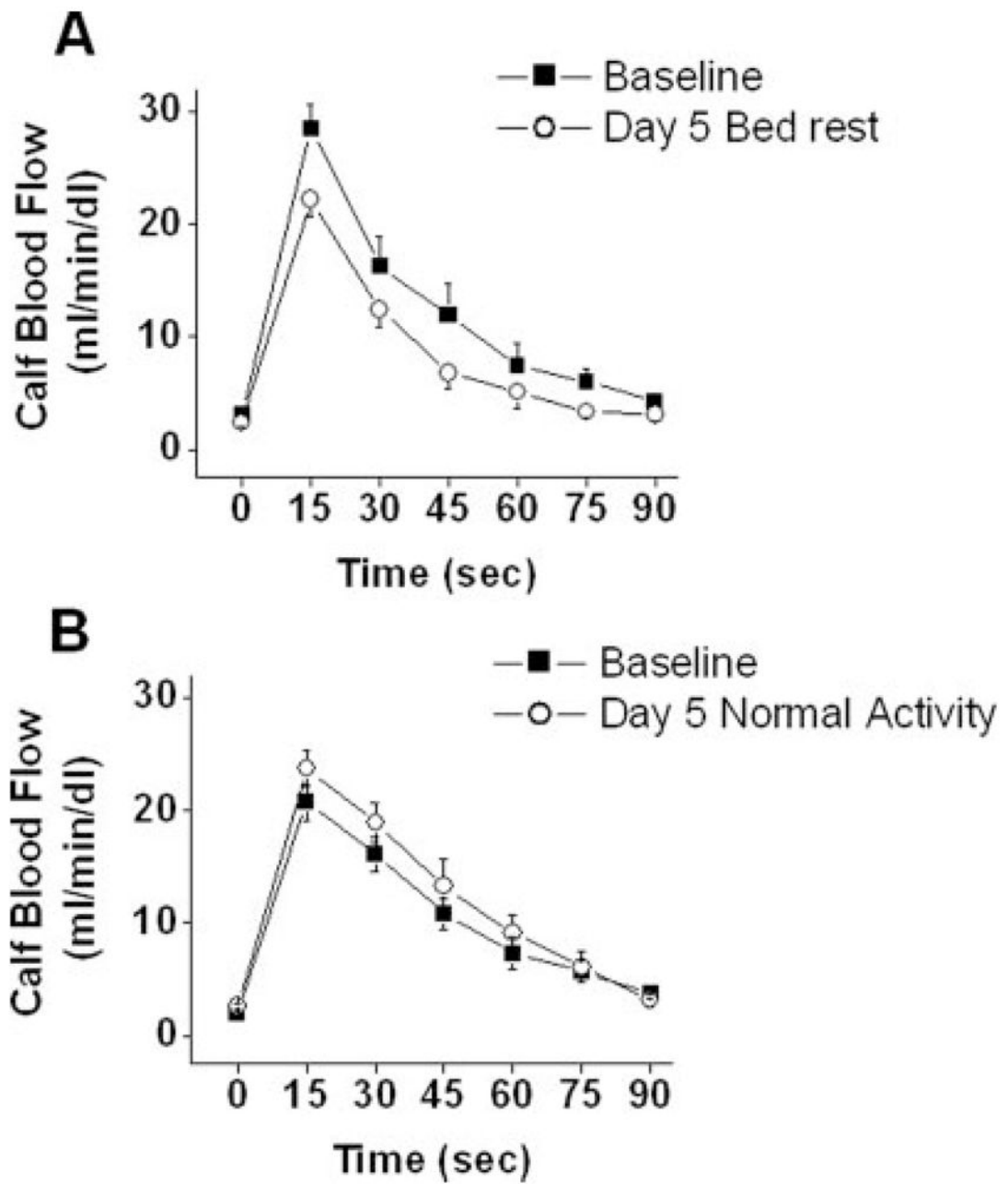
Figure 3.
Reactive hyperemia in the lower extremity assessed using venous occlusion plethysmography at baseline and after bed rest (A) or normal activity (B). Bed rest impaired peak and net hyperemic response (*$P<0.01$ and $P<0.05$ respectively, n=10). In active control subjects, there were no differences in peak or net hyperemic response (P=NS, n=9).

Table 1

Effects of Physical Inactivity on Metabolic Parameters

	Baseline Day 1	Inactive Day 5	*P* Value
Weight, kg	70.4±11.4	69.9±11.2	0.09
Total cholesterol, mg/dL	147±24	159±25	0.001
LDL cholesterol, mg/dL	83±24	90±24	0.07
HDL cholesterol, mg/dL	51±12	50±11	0.38
Triglycerides, mg/dL	69±21	93±30	0.001
Glucose, mg/dL	84.7±7.0	87.8±6.1	0.02
Insulin, μIU/mL	4.7±4.1	6.9±5.4	0.01
HOMA-IR	1.0±0.9	1.5±1.4	0.004
$ISI_{0,120}$, mg*L^2/mmol*mIU*min	32±7	28±5	0.008

Data are presented as mean±SD.

Table 2

Effects of Physical Inactivity on Brachial Artery Parameters and Blood Pressure

	Baseline Day 1	Bed Rest Day 3	Bed Rest Day 5	*P* Value
Systolic blood pressure, mm Hg	109±8	112±9	116±13	0.01
Diastolic blood pressure, mm Hg	64±8	66±6	68±7	0.21
Heart rate, bpm	64±8	64±10	64±8	0.93
Baseline diameter, mm	4.19±0.46	4.09±0.43	4.08±0.46	0.02
Flow-mediated dilation, mm	0.35±0.13	0.39±0.12	0.35±0.13	0.15
Flow-mediated dilation, %	8.5±3.4	9.7±3.4	8.7±3.5	0.14
Nitroglycerin-mediated dilation, % [*]	14.4±3.9	19.6±2.9	18.9±3.0	0.14
Flow-mediated dilation, mm/hyperemic flow velocity, mm [*] (100 [*] cm/s^{-1})	0.23±0.09	0.29±0.11	0.25±0.10	0.003
Baseline flow, mL/min	199±147	153±81	156±90	0.13
Baseline flow velocity, cm/s	23.1±2.9	18.8±1.6	19.3±1.6	0.14
Peak flow velocity, cm/s	157.4±7.4	137.4±6.1	141.2±5.5	0.01

Data are presented as mean±SD, *P* values for RM-ANOVA.

[*] n=7.

Table 3

Effects of Physical Inactivity on Biomarkers

	Baseline Day 1	Bed Rest Day 3	Bed Rest Day 5	*P* Value
C-reactive protein, mg/L	1.0±1.2	...	1.2±1.9	0.40
Interleukin-6, pg/mL	1.2±0.7	1.4±2.2	1.0±0.6	0.59
Tumor necrosis factor receptor-II, pg/mL	1870±305	1870±370	1880±360	0.95
Adiponectin, ng/mL	9540±2950	9150±3170	9220±3140	0.61

Data are presented as mean±SD. n=13–19.