Effects of Hyperinsulinemia on Sympathetic Responses to Mental Stress
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In a recent study, we could not find evidence to support the hypothesis that insulin activates the sympathetic nervous system (SNS) during a hyperinsulinemic glucose clamp procedure. Mental stress tests (MST), however, may be used to detect differences in blood pressure and SNS activity that are not present during baseline or resting conditions. In this study, we aimed to investigate the effects of hyperinsulinemia during glucose clamp on blood pressure and sympathetic responses to mental stress.

Borderline hypertensive but otherwise healthy 21-year-old men (n = 18) underwent 5 min of mental arithmetic stress testing (MST-1) before and at the end of 120 min of isoglycemic hyperinsulinemic glucose clamp (MST-2) with infusion rates of glucose and insulin kept constant. Insulin concentration increased from 119 ± 10 pmol/L to 752 ± 65 pmol/L. We observed highly significant increases in blood pressure and heart rate in response to MST, but neither insulin nor saline solution infusions affected these responses. During MST-1, norepinephrine increased by 461 ± 165 pmol/L (mean ± SEM) and epinephrine by 218 ± 76 pmol/L. During MST-2 the changes were 372 ± 112 pmol/L and 187 ± 60 pmol/L, respectively. The norepinephrine (P = .8) and epinephrine (P = .7) responses were unchanged by insulin. Thus, there were similar increases in blood pressure, heart rate, and plasma catecholamine concentrations in arterialized venous blood in response to MST despite the infusion of insulin. A possible time effect was excluded by including a saline solution control group (n = 7) that showed almost identical results.


KEY WORDS: Hyperinsulinemia, mental arithmetic stress test, sympathetic nervous system, catecholamines.

There is a well-established relationship between hypertension and reduced ability of insulin to regulate levels of glucose in blood. Activation of the sympathetic nervous system (SNS) by insulin has been proposed as one mechanism explaining the connection. Others have suggested that increased SNS activity could initiate insulin resistance. Increased SNS activity has been reported during glucose clamp examination and after oral carbohydrate ingestion. The influence of

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insulin on the SNS has been determined by measuring plasma catecholamine concentrations or by micro-neuroelectrography. Most studies have reported an increase in SNS activity over baseline. A common feature in many of these studies, however, is the lack of a control group. We recently showed that the glucose clamp procedure activated the SNS, even in the absence of hyperinsulinemia, ie, the clamp procedure per se increased SNS activity, and we disputed the hypothesis that hyperinsulinemia activates the SNS.

We have already shown that increased sympathetic reactivity to mental stress may be independent of resting blood pressure and baseline catecholamine concentration. Thus, by applying mental stress we may reveal important features in sympathetic function that are not detectable at baseline. Cardiovascular hyperreactivity to stress may be an important predictor of hypertension and perhaps be an early sign of increased sympathetic activity. In our previous study, insulin infusion did not alter baseline plasma catecholamine concentration compared to saline solution infusion. However, the effect of insulin on sympathetic reactivity to stress may differ from the effects on baseline conditions. In this study we have addressed this issue specifically.

MATERIALS AND METHODS

Subjects We examined 21-year-old Caucasian men recruited from medical examination records taken during the military draft procedure in the city of Oslo. Attending the draft procedure is compulsory, and only men with severe medical disorders do not take part. Therefore, the population was comprised of all healthy 18-year-old men in the Oslo area (approximately 3500 men yearly). Sitting blood pressure, heart rate, body weight, and height were recorded. No follow-up of the subjects was undertaken until this study was performed 3 years later.

We invited subjects who had blood pressure at or higher than 140/90 mm Hg on one occasion, (ie, the medical examination during the military draft procedure in 1991), to attend a cardiovascular risk-factor screening at Ullevaal Hospital. They underwent a thorough physical examination and blood chemistry testing, which included renal and liver function tests and urinalysis to exclude illness. They were all healthy and none used medication regularly. Despite elevated blood pressure at military enlistment (which they were not informed about), most of them were normotensive at baseline. From our experience, we knew that subjects recruited this way were more likely to have increased blood pressure responses to mental stress than their fellow participants.

The study was approved by the Regional Ethics Committee, and informed consent was obtained from each subject. All subjects fasted and refrained from smoking for at least 10 h, and abstained from alcohol for the last 24 h before the study.

Eighteen subjects participated in the insulin infusion group. Body mass index averaged 24 ± 0.7 kg/m², supine heart rate 66 ± 3 beats/min, supine blood pressure 137 ± 3/77 ± 2 mm Hg, and glucose disposal rate (GDR) 7.4 ± 0.7 mg/kg/min.

Sixteen subjects from the same population underwent identical procedures, except that insulin and glucose were substituted with matched quantities of saline solution. These subjects had a mean body mass index of 24 ± 0.6 kg/m², supine heart rate of 62 ± 2 beats/min, and supine blood pressure of 134 ± 3/74 ± 2 mm Hg. Seven of these men underwent the complete procedure including MST before and after clamp. These subjects had a mean body mass index of 23 ± 0.8 kg/m², supine heart rate of 63 ± 4 beats/min, and supine blood pressure of 142 ± 3/76 ± 2 mm Hg. The remaining (n = 9) subjects served as plain time controls with no intervention except the saline solution infusion. There were no changes in blood pressure, heart rate, or plasma catecholamine concentration in this subgroup at the time points when mental stress tests (MST) were otherwise carried out.

Laboratory Methods An antecubital vein on the right arm was cannulated with a short Teflon catheter (Venflon 17G, Viggo AB, Helsingborg, Sweden) and the right forearm was then placed in a heating sleeve (Thermal Vascular Dilatator, Sweutron AB, Veddesta, Sweden). The temperature was set at 52.0°C, and the right arm was used for sampling of arterialized venous blood. Heating the arm induced shunting of arterial blood to the veins, thus providing a sample that imitated arterial blood. An antecubital vein on the contralateral arm was also cannulated with a short Teflon catheter (Venflon 18G) for later infusion of insulin and glucose or saline solution. The subjects then rested supine for 20 min before baseline blood pressures and heart rates were recorded and blood samples were obtained.

The isoglycemic hyperinsulinemic glucose clamp technique was performed with a modification of the method by DeFronzo et al. Insulin was infused at a fixed rate of 1 mU/kg/min. This technique for measuring the GDR has a day-to-day coefficient of variation (CV) of <5% in our laboratory. We used an Accutrend (Mannheim Boehringer GmbH, Mannheim, Germany) to measure glucose concentration every 5 min during the glucose clamp procedure (within-run CV <2%). Insulin was measured by radioimmunoassay with a specific antibody from Linco Research (St. Louis, MO). The intraassay CV was <9% at all levels of insulin. Plasma catecholamine concentrations from arterialized venous blood were measured by the radioenzymatic technique of...
Peuler and Johnson\textsuperscript{16} as described.\textsuperscript{17} Blood pressure and heart rate were measured oscillometrically with an Omega 1000 Adult/Pediatric Blood Pressure Recorder (INVIVO Research Laboratories, Tulsa, OK) as reported.\textsuperscript{18}

We applied a strictly standardized mental arithmetic stress test (MST-1) immediately before and at the end of 120 min of clamp (MST-2). Participants were required to repeatedly subtract 13 from 1079 for 5 min. Sound (2 Hz) from a metronome disturbed the procedure and increased the stress. The CV was calculated from the formula $CV = \sqrt{\frac{\sum (D^2)/2n}{X}}$ in which $D$ = difference between corresponding pairs of observations, $n$ = number of subjects, and $X$ = mean value of the response indicator. When comparing the responses in blood pressure and heart rate between two MST, we found a CV of 8% and 9.5%, respectively. During MST-2 the infusions of glucose and insulin continued at constant rates (Figure 1). Blood pressure, heart rate, and plasma catecholamine concentrations were measured before (basal) the test, during announcement of the test, 1 min after announcement of the test, four times during the test with 100-sec intervals, and after 10 min of recovery. The total duration of the MST was 7 min, which included 2 min of anticipation during which much of the increase in SNS activity occurred.\textsuperscript{12} Each subject met the same examiner during the first and the second test.

**Statistical Analysis** The data were analyzed with the SPSS V6.1 statistical package (SPSS Inc., Chicago, IL). Data are presented as the mean $\pm$ SEM. Between-group differences were tested by the two-tailed Student’s $t$ test after using the Kolmogorov-Smirnov test to check for normal distribution of data. For data not normally distributed, the nonparametric Wilcoxon signed rank test was used to evaluate differences. The changes in cardiovascular and catecholamine responses during MST-1 and MST-2 were also compared by calculating the area under the curve for the responses (ie, differences between maximal and basal values). The area under the curve was calculated from announcement to the end of MST (ie, a measure of the reactivity). Because responses were expected to increase and not decrease during MST, one-tailed tests were used for within-group responses during MST. This presumption was based on experience from previous studies using MST in subjects from the same population.\textsuperscript{9,12} For correlation analysis, the Pearson correlation coefficient ($r$) was estimated. A $P$ value of .05 was considered the limit of statistical significance.

**RESULTS**

**Effect of Insulin Infusion During Glucose Clamp and Saline Solution on Blood Pressure, Heart Rate, and Plasma Catecholamine Concentrations at Rest** Two hours of hyperinsulinemic isoglycemic glucose clamp or saline solution infusion did not change blood pressure, heart rate, or plasma epinephrine concentrations (Table 1). Both interventions induced moderate, similar, and statistically significant increases in plasma norepinephrine concentrations (Table 1). A small baseline difference in norepinephrine (Table 1) persisted through the infusions (Table 1).

**Effect of Insulin and Saline Solution Infusion on Blood Pressure Responses to MST** The blood pressure responses to MST were highly significant both before and during the end of the infusion ($P < .01$). Insulin infusion did not significantly change the area under the curve for systolic blood pressure ($P = .1$) or diastolic blood pressure ($P = .6$) responses to MST (Figure 2). There were no changes in the area under the curve for systolic blood pressure ($P = .9$) and diastolic blood pressure ($P = .2$) responses to MST when saline solution was infused (Figure 2). There was no difference in systolic blood pressure or diastolic blood pressure responses assessed as area under the curve between the insulin clamp group and the saline solution control group either before ($P = .5, P = .7$, respectively) or after the infusion ($P = .6, P = .7$, respectively) was performed.

The maximal systolic blood pressure responses were almost identical before and after the infusions with no differences between insulin and saline solution groups (Table 2). The maximal diastolic blood pressure responses to MST were, however, moderately but significantly attenuated in response to the infusions (Table 2) with no differences between the two groups. There were also rather strong correlations between the maximal blood pressures during MST before and at the end of the infusions, with $r$ values between 0.53 and 0.87 in both groups (Table 3).
Effect of Insulin and Saline Solution Infusion on Heart Rate Responses to MST

The heart rate responses to MST were highly significant both before and at the end of the infusions \((P < .001)\). However, neither insulin infusion \((P = .6)\) nor saline solution \((P = .6)\) changed the area under the curve for heart rate responses to MST (Figure 2).

There was no difference in heart rate responses assessed as area under the curve between the insulin clamp group and the saline solution control group either before \((P = .5)\) or after the infusions \((P = .9)\).

The maximal heart rate responses were the same before and after the infusions with no difference between insulin and saline solution groups (Table 2). Maximal heart rate during MST before and at the end of infusion showed a strong correlation in both groups (Table 3).

Effects of Insulin and Saline Solution Infusion on Plasma Catecholamine Responses to MST

The increases in plasma catecholamine concentrations during MST before and at the end of the infusions were statistically significant \((P < .05)\). However, insulin infusion did not significantly change the area under the curve for plasma norepinephrine \((P = .7)\) or plasma epinephrine \((P = .9)\) responses to MST (Figure 3). Neither was there any change in the area under the curve for plasma norepinephrine \((P = .7)\) nor plasma epinephrine \((P = .8)\) responses to MST when saline solution was infused (Figure 3).

There was no difference in plasma catecholamine responses (area under the curve) between the insulin clamp group and the saline solution control group either before \((P = .6, P = 1.0,\) respectively) or after the infusions \((P = .5, P = .4,\) respectively) were performed.

The norepinephrine responses were \(461 \pm 165\) pmol/L vs \(572 \pm 112\) pmol/L \((P = .8)\) and the epineph-

![Figure 2](attachment:image.png)
rine responses were 218 ± 76 pmol/L v 187 ± 60 pmol/L (P = .7) in the insulin group. In the controls, norepinephrine increased by 459 ± 256 pmol/L v 632 ± 257 pmol/L (P = .7) and epinephrine by 167 ± 78 pmol/L v 261 ± 80 pmol/L (P = .2). Norepinephrine recovered somewhat slower than the other indicators (Figure 3); however, there were no significant group differences (P = .1, P = .6), or differences with or without insulin (P = .3, P = .5).

The correlations between the maximal responses to MST before and after the infusions were significant except for that of plasma norepinephrine in the saline solution group (Table 3).

**DISCUSSION**

The main finding in this study is that blood pressure, heart rate, and plasma catecholamine responses to MST performed before and during hyperinsulinemic glucose clamp were almost identical; ie, the blood pressure and SNS responses to mental arithmetic stress were unaffected by acute hyperinsulinemia.

An acute increase in plasma insulin during hyperinsulinemic glucose clamp has been claimed to activate the SNS as determined by plasma norepinephrine concentrations and microneurography. Insulin is considered to be sympathoexcitatory and thereby con-

**TABLE 2. EFFECT OF INSULIN INFUSION DURING GLUCOSE CLAMP AND SALINE ON BLOOD PRESSURE AND HEART RATE RESPONSES TO MENTAL ARITHMETIC STRESS TEST (Δ VALUES BETWEEN MAXIMAL LEVELS DURING STRESS AND LEVELS AT REST)**

<table>
<thead>
<tr>
<th></th>
<th>Insulin Infusion and Isoglycemic Glucose Clamp (n = 18)</th>
<th>Saline Infusion (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MST-1         MST-2         P</td>
<td>MST-1         MST-2         P</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>23 ± 3        21 ± 3        .50</td>
<td>22 ± 4        19 ± 3        .44</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>14 ± 2        10 ± 2        .05*</td>
<td>14 ± 2        8 ± 3         .02*</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>31 ± 3        30 ± 5        .50</td>
<td>33 ± 3        31 ± 4        .60</td>
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Mean ± SEM.

* Statistically significant decrease in response (in both groups).

SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate.

**TABLE 3. REGRESSION COEFFICIENTS FOR MAXIMAL BLOOD PRESSURE, HEART RATE, AND PLASMA CATECHOLAMINE CONCENTRATION BETWEEN TWO MENTAL ARITHMETIC STRESS TESTS; THE FIRST TEST BEFORE INFUSIONS AND THE SECOND TEST AT THE END OF 120 MIN OF INSULIN OR SALINE INFUSION**

<table>
<thead>
<tr>
<th></th>
<th>Insulin Infusion and Isoglycemic Glucose Clamp (n = 18)</th>
<th>Saline Infusion (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r       P</td>
<td>r       P</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>0.78   .0001</td>
<td>0.66   .08†</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>0.63   .005</td>
<td>0.73   .04</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>0.86   .0001</td>
<td>0.87   .005</td>
</tr>
<tr>
<td>NE (pmol/L)</td>
<td>0.67   .003</td>
<td>0.58   .20†</td>
</tr>
<tr>
<td>Epi (pmol/L)</td>
<td>0.53   .03</td>
<td>0.79   .04</td>
</tr>
</tbody>
</table>

† Not statistically significant.

SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; NE, norepinephrine; Epi, epinephrine.
tributes to the development of hypertension. The mechanism for this is unclear, but both a baroreflex response to the vasodilator effect of insulin or a direct central neural action have been considered. On the other hand, Masuo et al recently published a longitudinal study in which they concluded that increased SNS activity seems to precede the emergence of hyperinsulinemia, supporting the hypothesis that increased SNS activity leads to insulin resistance and not vice versa.

Recently we studied the effects of the glucose clamp procedure on SNS activity. We included a control group receiving saline solution instead of insulin and found no significant change in blood pressure, heart rate, or plasma epinephrine concentration during clamp or between the groups. However, we found a significant but identical increase in plasma norepinephrine in both groups during the clamp. Thus, in our hands insulin does not seem to increase the plasma catecholamine concentrations; but the increase in norepinephrine is rather a response to the clamp procedure per se. In this study, we observed the same effects of the clamp procedure; ie, unchanged blood pressure and heart rate, an increase in norepinephrine concentration both during insulin and saline solution infusion, and a tendency toward a decrease in epinephrine concentration.

The mental arithmetic stress test has been used in numerous studies and induces a sharp increase in SNS activity accompanied by an increase in cardiac output, a redistribution of blood flow from viscera to skeletal muscle, and reduced peripheral resistance. The cardiovascular and neuroendocrine responses to MST are similar to the so-called alarm-defense reaction.

To our knowledge, no other studies have examined the effect of insulin infusion on SNS responsiveness to MST. If insulin per se enhances SNS responsiveness, we would expect an increased response to mental stress under the hyperinsulinemic conditions. Our results do not show this, thus lending no support to the concept that insulin enhances SNS activity in the development of insulin resistance and hypertension.

Rostrup et al recently reported that young men similar to those in this study had a specific cardiovascular hyperreactive response to MST but not to the cold-pressor test. Similarly, Jern found that MST was a more powerful stimulus of SNS activity than interviews, video-displayed medical information, noise, and anxiety-provoking situations for both normotensive and borderline hypertensive young men. Our subjects had rather impressive responses to MST, with changes in blood pressure of 21/12 and heart rate of 31 beats/min. As already stated, this may be because they were chosen from a group of young men who had rather high blood pressures at military drafting and high normal blood pressures in the control situation. These men may be regarded as hyperreactive and possibly prone to develop hypertension later in life. Being hyperreactive did not mean they cannot be considered healthy because their basal blood pressures in the laboratory were almost normal and none of the subjects took medication regularly. That insulin did not increase SNS responses in this hyperreactive group is interesting by itself because one might postulate that among hyperreactive subjects there should be a tendency to react in a specific manner, ie, with increased SNS response to insulin.

Many authors have shown a high test-retest correlation, ie, good reproducibility during mental stress. Jern et al showed that MST is highly reproducible with regard to activation levels attained during stress for heart rate, blood pressure, and subjective stress rating. The CV for these measures ranged between 3% and 6% and there were no significant differences between levels of activation during the two tests for any of the response variables. They found a lower reproducibility for catecholamine responses, possibly due to venous sampling for catecholamine testing. We increased the serum insulin level between the two MST in the clamp group in this study, which might have influenced our results. However, the SNS responses, ie, maximal changes in heart rate, blood pressure, plasma catecholamine concentrations, and the total responses calculated as area under the curve for all parameters were similar during the two stress tests.

There was a trend toward lower responses to the second MST both in the insulin group and the control group, though the responses were significant only for diastolic blood pressure. Although other authors have demonstrated good reproducibility for MST, we cannot rule out that the marginal differences in maximal diastolic blood pressure response were due to a time (order-of-treatment) effect. Theoretically, a weak effect of insulin on SNS responses may have been canceled out by a time effect. This time effect, however, was present in both groups and there was no difference in the integrated responses assessed as area under the curve with and without insulin.

In this study, we measured plasma catecholamine concentrations in arterialized venous blood to describe SNS responses. Measurements of muscle sympathetic activity (MSNA) by microneurography recordings is another way of assessing SNS activity. However, Wallin et al showed a strong positive correlation between SNS activity during microneurography and venous plasma levels, total norepinephrine spillover, and regional norepinephrine spillover. Recently Grassi et al showed that an alert reaction in seven mild untreated hypertensives was accompanied by a significant decrease in MSNA from the peroneal nerve, whereas skin sympathetic nerve activity, heart
rate, and blood pressure increased. The reduction in MSNA most probably represents reflex influences triggered by the rise in blood pressure. Plasma catecholamine concentrations may thus be a more integrated measure of SNS activity during MST than isolated nerve recording.

We have studied healthy though hyperreactive young men and hence cannot easily generalize to a wider population. The effects of acute hyperinsulinemia might also be different from those of chronic hyperinsulinemia, and we have not used other techniques that alter insulin responses, such as steady-state plasma glucose or the minimal model.

Although GDR was not measured in the saline solution control group, all subjects were drawn from the same population previously assessed to have GDR on the same level as the insulin infusion group in the study. Thus, group differences in GDR are unlikely to be present.

In conclusion, despite a sevenfold increase in insulin concentration, the blood pressure and SNS responses to the mental arithmetic stress test were unaffected. Acute hyperinsulinemia does not seem to interfere with the sympathetic responses in healthy young men. This result adds to our recent finding that the increase in plasma norepinephrine during glucose clamp is caused by the procedure itself and not by hyperinsulinemia. Thus, we find no evidence supporting the hypothesis that insulin increases blood pressure or activates the SNS.

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