# Glucose Disposal Rates Calculated From 60- to 90-Minute Isoglycemic Hyperinsulinemic Glucose Clamp Correlate With Cardiovascular Risk Factors in Borderline Hypertensive Young Men

H.M. Reims, A. Høieggen, E. Fossum, M. Rostrup, I. Eide, and S.E. Kjeldsen

**The hyperinsulinemic glucose clamp is generally performed for at least 120 minutes, due to assumptions of steady-state. We were interested in relationships between glucose disposal rate (GDR) and cardiovascular risk factors, rather than a standard measure of insulin sensitivity per se. Therefore, we analyzed 120-minute clamps performed on borderline hypertensive, but otherwise healthy young men (n = 19). GDR was calculated at different time points and related to baseline cardiovascular risk factors and responses to a mental stress test (MST). The 60-, 90-, and 120-minute GDR correlated significantly with serum high-density lipoprotein (HDL) cholesterol ($r = .59$, $r = .50$, and $r = .53$, respectively), heart rate (HR) during MST ($r = -.65$, $r = -.64$, and $r = -.58$, respectively) and plasma epinephrine (Epi) ($r = -.55$, $r = -.58$, and $r = -.56$, respectively) and norepinephrine (NE) ($r = -.52$, $r = -.49$, and $r = -.48$, respectively) 1 minute after announcement of the MST (all $P < .05$). Although not statistically significant at all time points, similar relationships were observed between GDR and resting HR, systolic blood pressure (BP) at rest and during mental stress, body mass index (BMI), serum total cholesterol (Chol), serum triglycerides (TG), and blood hemoglobin (HgB), with remarkable consistency from about 40 to 50 minutes onwards. HDL cholesterol and Epi remained independent in stepwise multiple regression analyses with the 60-, 90-, and 120-minute GDR as dependent variables (all $P < .05$). We suggest that 60- to 90-minute glucose clamps may provide information about the relationship between insulin sensitivity and various cardiovascular risk factors in borderline hypertensive young caucasian men.**


INSULIN RESISTANCE is related to high blood pressure (BP),[1,2] and may be involved in the etiology of non–insulin-dependent diabetes mellitus (NIDDM), hypertension, disorders of lipoprotein metabolism, and atherosclerotic cardiovascular disease.[3,4] The glucose clamp technique[5,6] is widely used and provides the most accurate measure of insulin sensitivity, the inverse of insulin resistance, in vivo.[7,8] Using a modification of this technique,[9,10] we have assessed insulin sensitivity by calculating glucose disposal rate (GDR) during the last 20 minutes[10,11] or 60 minutes[12] of a 120-minute clamp. When applying this method to various subsets of healthy young men with high screening BP, we have previously found correlations between insulin resistance and other cardiovascular risk factors, including serum lipid levels and body mass index (BMI), as well as BP, heart rate (HR), and plasma catecholamine responses during mental stress.[9-15] The rationale for studying young, borderline hypertensive men is that among these subjects, we expect a higher prevalence of cardiovascular risk factors than in the general population,[16-19] implying that these subjects are also suitable for studies of relationships between risk factors. Since the glucose clamp procedure is relatively time-consuming and demanding, we have been interested in detecting an earlier cut-off point for assessing insulin sensitivity and its relation to other variables. Therefore, the purpose of this study was to explore the relationship between glucose disposal at early stages of the clamp and various other parameters, and to compare this information with that drawn from the end of a 120-minute clamp.

In a retrospective analysis of existing data, we recently showed that in series of 120-minute glucose clamps performed by independent investigators on healthy, young men with high screening BP ("borderline hypertensive"), the correlation between GDR and fasting plasma insulin was statistically significant and practically unchanged whether GDR was calculated after 60, 90, or 120 minutes.[20]

There were also significant correlations between GDR and a number of other cardiovascular risk factors. In the present study, we therefore extended the retrospective analysis of this group by including parameters that tended to correlate with the 120-minute GDR (ie, our standard measure of insulin sensitivity). As GDR can be calculated at any time point during clamp, we were interested to see whether a shorter clamp might have given the same information about such relationships as the standard length clamp. The aim was therefore to explore the time aspect of relationships between GDR and BP, HR, and plasma catecholamine levels at rest and during mental stress, as well as serum lipids and blood hemoglobin (Hgb) in one series of 120-minute clamps (n = 19).

## SUBJECTS AND METHODS

### *Subjects*

We examined caucasian men recruited from the records based on medical examination during the military draft procedure in the city of Oslo, where sitting BP, HR, body weight, and height were recorded. The population comprises all healthy 18- to 19-year-old men in the Oslo area (yearly approximately 3,500 to 4,000 men).

We invited men with BP at or higher than 140/90 mm Hg on one occasion, ie, the military draft procedure in 1991, to attend a cardiovascular risk factor screening at Ullevaal Hospital. They were examined with the isoglycemic hyperinsulinemic glucose clamp technique in 1994. Despite borderline BP elevation at the military enlistment, all subjects were healthy and none used regular medication. There were 5 smokers and 14 nonsmokers. All subjects fasted and refrained from smoking for the preceding 10 hours and abstained from alcohol for the preceding 24 hours before the study. Mean age was 21.7 years (all aged 21 to 22), body mass index (BMI) 24.4 kg/m$^2$ (SD 3.2), fasting serum

*From the Departments of Cardiology and Nephrology, Ullevaal University Hospital, Oslo, Norway.*

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*Address reprint requests to Henrik Reims, MD, Department of Cardiology, Ullevaal University Hospital, N-0407 Oslo, Norway.*



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glucose 4.9 mmol/L (SD 0.5), fasting plasma insulin 121 pmol/L (SD 46), serum total cholesterol (Chol) 4.4 mmol/L (SD 0.7), serum high-density lipoprotein (HDL) cholesterol 1.0 mmol/L (SD 0.2), serum triglycerides (TG) 1.3 mmol/L (SD 0.6), and blood Hgb 15.0 g/100mL (SD 0.8). Mean systolic BP (SBP) was 135 (SD 17) and 151 mm Hg (SD 19) at rest and during mental stress, respectively, diastolic BP (DBP) 76 (SD 9) and 84 mm Hg (SD 11), HR 66 (SD 12) and 86 beats/min (SD 24), plasma epinephrine (Epi) 321 (SD 287) and 385 pmol/L (SD 310), and plasma norepinephrine (NE) 1,023 (SD 342) and 1,663 pmol/L (SD 719). The hemodynamic and sympathetic responses to mental stress have previously been published.[21]

### *The Isoglycemic Hyperinsulinemic Glucose Clamp*

We performed the isoglycemic hyperinsulinemic clamp as previously described.[9,10,20] In short, an antecubital vein on the right arm was cannulated and the forearm was placed in a heating sleeve set on 52°C, and was used for sampling arterialized venous blood. An antecubital vein on the left arm was cannulated for infusion of insulin and glucose. The fasting glucose level was determined 20 minutes after the heating sleeve was adapted, as the average of 3 bedside measurements. Insulin was infused at a fixed rate of 1 mU/min/kg body weight (6 pmol/min/kg body weight). Glucose infusion (240 mg/mL) was started after 5 minutes. Blood glucose level was determined every 5 minutes and was clamped at the subject's fasting level (isoglycemia) by adjustment of the rate of glucose infusion according to the results.

Isoglycemia was maintained for 120 minutes, and GDR was calculated from the amount of glucose infused during the last 20 minutes as (glucose concentration in infused solution × mean glucose infusion rate)/body weight. GDR thus corresponds to M,[6] ie, whole body glucose uptake, without the correction for urinary glucose loss and space correction. This technique for measuring insulin sensitivity has a day-to-day coefficient of variation (CV) of 5% in our laboratory.[9,10] We then calculated GDR during 20-minute periods with 5-minute intervals from 30 to 60 minutes, and with 10-minute intervals from 60 to 120 minutes of the clamp.

### *Mental Arithmetic Stress Test*

We applied a standardized mental arithmetic stress test (MST) immediately at the end of the 120-minute glucose clamp examination. Glucose and insulin infusions were continued at constant rates during MST. The subjects were asked to subtract the number 13, starting with the number 1,079, continuously for 5 minutes, while a metronome was used for distraction, and they were informed about any miscalculation. They were not informed about the test until 2 minutes before it started. Much of the increase in sympathetic nervous system (SNS) activity occurs during these 2 minutes of anticipation.[22] BP and HR were measured immediately before announcement of the test, 1 minute after announcement and after 3 to 4 minutes during the test. Plasma catecholamine concentrations were measured during announcement of the test, 1 minute after announcement, at the beginning of the test, after 3 to 4 minutes during the test, and at the end of the test. The within-day CV for BP and HR responses during MST are 8% and 9.5%, respectively, in our laboratory.[21]

### *Laboratory Methods*

Fasting serum glucose and lipid concentrations were measured with a Cobas Integra (Roche, Basel, Switzerland). Hgb concentration was measured with a Technicon H2 (Swords Co, Dublin, Ireland). Blood glucose concentration during clamp was measured with an Accutrend (Boehringer Mannheim, Mannheim, Germany). Insulin was measured by radioimmunoassay using a specific antibody from Linco Research (St Louis, MO), with an intra-assay CV of less than 9% at all levels. Plasma catecholamine concentrations from arterialized venous blood were measured by the radioenzymatic technique of Peuler and Johnson,[23] as previously described.[24] BP and HR at baseline and during MST were measured oscillometrically with an Omega 1000 Adult/Pediatric Blood Pressure Recorder (INVIVO Research Laboratories, Tulsa, OK), previously evaluated in our laboratory.[25]

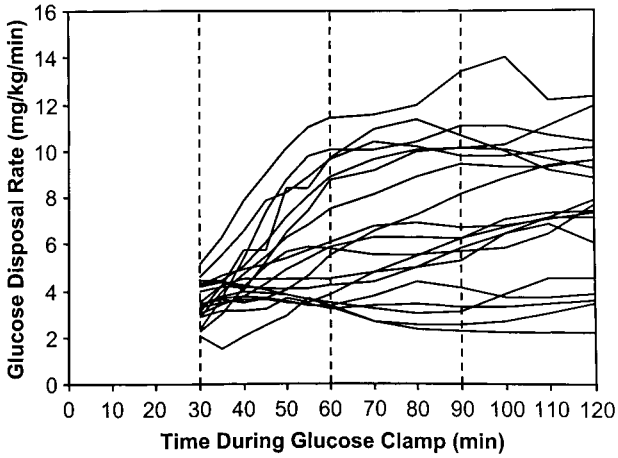


**Fig 1. GDR for individual subjects calculated from the last 20 minutes with 5-minute intervals from 30 to 60 minutes, and with 10-minute intervals from 60 to 120 minutes during glucose clamp.**

### *Statistical Analysis*

The data were analysed using the statistical package SPSS version 10.0 for Windows (SPSS Inc, Chicago, IL). The GDRs at the chosen time points were compared in a repeated-measures analysis, where within-subjects contrasts were analyzed with the 120-minute GDR as the reference category. Pearson correlation coefficients ($r$) were calculated after testing for normality with the Kolmogorov-Smirnov test. Spearman's rank correlation coefficient ($r_s$) was used for non-normally distributed data. This was the case for HR 1 minute after announcement of MST (HR-A) and during mental stress (HR-MST), and Epi at all times. Ninety-five percent confidence intervals (CI) for $r$ and $r_s$ values were calculated according to Altman and Gardner.[26] Forward stepwise multiple regression analyses were performed with the 60-, 90-, and 120-minute GDR, respectively, as the dependent variable. Analyses were done with 3 sets of independent variables: all candidate variables, only those variables that correlated significantly with all 3 GDRs, and a selection of variables with a documented relation to insulin sensitivity. The probability for the F-value of .05 was used as the limit for entry and .10 for removal of variables. The regression coefficients ($b$), corresponding $P$ values, and adjusted $R^2$ are reported. $P$ values of less than .05 were considered statistically significant.

## RESULTS

### *Relations Between GDR at Different Time Points*

In a repeated-measures analysis, all GDRs calculated from 30 through 80 minutes were significantly lower than the 120-minute GDR, while the 90-, 100-, and 110-minute GDRs were not ($P = .073$, $P = .235$, and $P = .288$, respectively). The correlation coefficients ($r$ values) between the 60- and 120-minute GDR, and between the 90- and 120-minute GDR were 0.87 ($P < .001$) and 0.94 ($P < .001$), respectively. When curves for GDR of individual subjects are plotted together (Fig 1), they stay in a fairly constant rank order during the second half of the clamp.

**Table 1. Correlation Coefficients Between GDR at Different Time Points and Cardiovascular Risk Factors**

| | GDR 60 min | | | GDR 90 min | | | GDR 120 min | | |
|---|---|---|---|---|---|---|---|---|---|
| | *r* or $r_s$ | 95% CI | *P* | *r* or $r_s$ | 95% CI | *P* | *r* or $r_s$ | 95% CI | *P* |
| Baseline | | | | | | | | | |
| Hgb | −0.58 | (−0.82-−0.15) | .012 | −0.42 | (−0.74-0.05) | .080 | −0.33 | (−0.69-0.16) | .180 |
| BMI | −0.47 | (−0.76-−0.03) | .040 | −0.42 | (−0.73-0.05) | .076 | −0.46 | (−0.75-0.00) | .049 |
| Chol | −0.35 | (−0.70-0.12) | .138 | −0.37 | (−0.70-0.11) | .125 | −0.48 | (−0.77-−0.03) | .038 |
| HDL cholesterol | 0.59 | (0.19-0.83) | .007 | 0.50 | (0.06-0.78) | .029 | 0.53 | (0.10-0.79) | .020 |
| TG | −0.41 | (−0.73-0.05) | .081 | −0.39 | (−0.71-−0.08) | .103 | −0.46 | (−0.76-−0.01) | .046 |
| SBP | −0.49 | (−0.77-−0.05) | .033 | −0.36 | (−0.70-0.12) | .134 | −0.30 | (−0.66-0.18) | .122 |
| HR | −0.42 | (−0.73-0.04) | .073 | −0.44 | (−0.74-0.02) | .062 | −0.39 | (−0.72-0.07) | .095 |
| Glucose clamp and mental stress | | | | | | | | | |
| SBP-120 | −0.55 | (−0.80-−0.12) | .015 | −0.43 | (−0.74-0.03) | .065 | −0.44 | (−0.74-0.02) | .062 |
| SBP-A | −0.58 | (−0.82-−0.16) | .010 | −0.50 | (−0.78-0.06) | .028 | −0.41 | (−0.73-0.05) | .080 |
| HR-A | −0.40 | (−0.72-0.07) | .090 | −0.55 | (−0.80-−0.12) | .016 | −0.55 | (−0.80-−0.13) | .015 |
| HR-MST | −0.65 | (−0.85-−0.28) | .003 | −0.64 | (−0.85-−0.26) | .003 | −0.58 | (−0.82-−0.16) | .010 |
| Epi-D | −0.46 | (−0.76-0.01) | .062 | −0.49 | (−0.78-0.03) | .044 | −0.52 | (−0.79-−0.07) | .034 |
| Epi-A | −0.55 | (−0.81-−0.11) | .019 | −0.58 | (−0.82-−0.15) | .012 | −0.56 | (−0.82-−0.13) | .010 |
| NE-A | −0.52 | (−0.79-−0.09) | .027 | −0.49 | (−0.77-−0.05) | .038 | −0.48 | (−0.77-−0.04) | .042 |

NOTE. See Statistics section for definition of *r* and $r_s$.

Abbreviations: GDR, glucose disposal rate; Hgb, blood hemoglobin; BMI, body mass index; Chol, serum total cholesterol; HDL cholesterol, serum high-density lipoprotein cholesterol; TG, fasting serum triglycerides; SBP, systolic blood pressure; HR, heart rate; SBP-120, SBP after 120 minutes of clamp; SBP-A, SBP 1 minute after announcement of mental stress test (MST); HR-A, heart rate 1 minute after announcement of MST; HR-MST, heart rate during MST; Epi-D, plasma epinephrine during announcement of MST; Epi-A, plasma epinephrine 1 minute after announcement of MST; NA-A, plasma norepinephrine 1 minute after announcement of MST.

*Correlations Between GDR at Different Time Points and Baseline Cardiovascular Risk Factors*

GDR at 60 minutes correlated significantly with Hgb, BMI, HDL cholesterol, and SBP. GDR at 90 minutes correlated significantly with HDL cholesterol, and GDR at 120 minutes correlated significantly with BMI, Chol, HDL cholesterol, and TG. There were nearly statistically significant correlations between HR at baseline and GDR at 60, 90, and 120 minutes, respectively. The correlation coefficients between GDR at 60, 90, and 120 minutes and these variables are given in Table 1, and the relation of the *r* values (not always statistically significant) to time during clamp is shown in Fig 2.

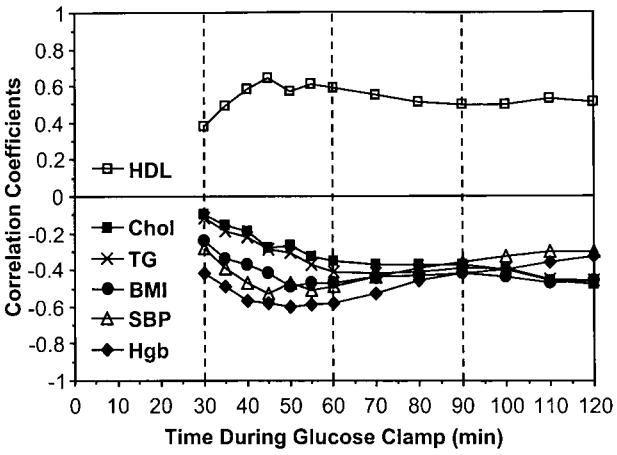


**Fig 2. Correlation coefficients between GDR at different time points and baseline cardiovascular risk factors. Markers indicate time points for calculation of GDR and the correlation coefficients.**

*Correlations Between GDR and BP and HR During Glucose Clamp and Mental Stress*

GDR at 60 minutes correlated significantly with SBP after 120 minutes of clamp (SBP-120) and 1 minute after announcement of MST (SBP-A), and with HR-MST. GDR at 90 minutes correlated significantly with SBP-A, HR-A, and HR-MST. GDR at 120 minutes correlated significantly with HR-A and HR-MST. The correlation coefficients between GDR at 60, 90, and 120 minutes and these variables are given in Table 1, and the relation of the *r* values (not always statistically significant) to time during clamp is shown in Fig 3.

*Correlations Between GDR and Plasma Catecholamine Levels During Mental Stress*

GDR at 60 minutes correlated significantly with Epi and NE 1 minute after announcement of MST (Epi-A and NE-A, respectively), GDR at 90 minutes with Epi during announcement of MST (Epi-D), Epi-A, and NE-A, and GDR at 120 minutes with Epi-D, Epi-A, and NE-A. GDR did not correlate significantly with plasma catecholamines during the arithmetic test itself. The correlation coefficients between GDR at 60, 90, and 120 minutes and these variables are given in Table 1, and the relation of the *r* values (although not always statistically significant) to time during clamp is shown in Fig 4.

*Regression Analyses*

The 60-, 90-, and 120-minute GDR were entered as dependent variables in forward stepwise multiple regression analyses. When all candidate independent variables were included, only Epi-A ($b$ = -0.070, $P$ = .030) and Hgb ($b$ = -1.622, $P$ = .043) remained significant for the 60-minute GDR ($R^2$ = 0.404), while only SBP-A ($b$ = -0.131, $R^2$ = 0.232, $P$ = .034)

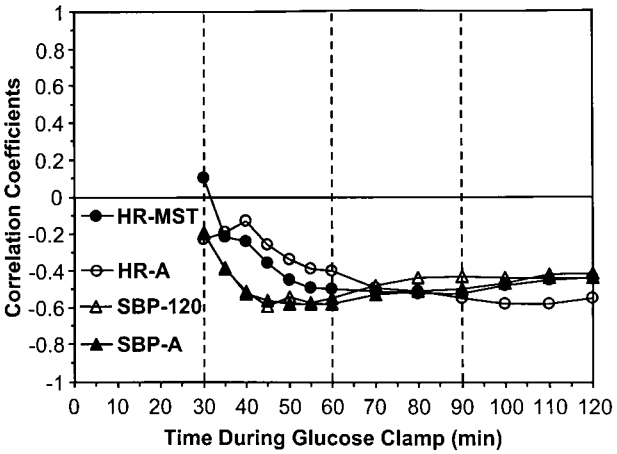


**Fig 3. Correlation coefficients between GDR at different time points and BP and HR levels during clamp and MST. Markers indicate time points for calculation of GDR and the correlation coefficients. HR-A, indicates heart rate 1 minute after announcement of MST; HR-MST, heart rate during MST; SBP-120, systolic blood pressure after 120 minutes of glucose clamp (before announcement of MST); SBP-A, systolic blood pressure 1 minute after announcement of MST.**

remained significant for the 90-minute GDR, and SBP-120 ($b$ = -0.172, $R^2$ = 0.228, $P$ = .035) for the 120-minute GDR.

When HDL cholesterol, HR-MST, Epi-A, and NE-A (the only significant correlates of GDR at all 3 chosen time points, see Table 1) were entered, both HDL cholesterol and Epi-A remained independent for the 60-minute GDR ($b$ = 12.216, $P$ = .001 and $b$ = -0.029, $P$ = .002, respectively, $R^2$ = 0.594), the 90-minute GDR ($b$ = 13.411, $P$ = .003 and $b$ = -0.036, $P$ = .002, respectively, $R^2$ = 0.539), and the 120-minute GDR ($b$ = 12.310, $P$ = .005 and $b$ = -0.028, $P$ = .010, respectively, $R^2$ = 0.463).

When BMI, HDL cholesterol, Chol, TG, SBP-A, HR-A, Epi-A, and NE-A were entered, BMI ($b$ = -0.322, $P$ = .028), HDL ($b$ = 8.959, $P$ = .008), and Epi-A ($b$ = -0.032, $P <$ .0005) remained independently associated with the 60-minute GDR ($R^2$ = 0.695). Chol ($b$ = -1.772, $P$ = .037), HDL cholesterol ($b$ = 9.248, $P$ = 0.031), and Epi-A ($b$ = -0.042, $P <$ .0005) remained independent with the 90-minute GDR as the dependent variable ($R^2$ = 0.642). Similarly, with the 120-minute GDR as the dependent variable, Chol ($b$ = -2.042, $P$ = .010), HDL cholesterol ($b$ = 7.513, $P$ = .045), and Epi-A ($b$ = -0.035, $P$ = .001) remained the only independent variables ($R^2$ = 0.650).

## DISCUSSION

We found that cardiovascular risk factors, including BP and serum lipids, correlate with GDR calculated at 60, 90, and 120 minutes of hyperinsulinemic glucose clamping. Although not statistically significant at all time points, the correlation coefficients between GDR and the other cardiovascular risk factors showed remarkable consistency from about 40 to 50 minutes onwards. As we have previously shown,[20] this was also the case for correlation coefficients between GDR at different time points and fasting plasma insulin, which showed a similar pattern when plotted against time. The explanation seems to be that GDR from 60 minutes onward correlates rather strongly with GDR at 120 minutes. Also, the difference between GDR at 90 minutes and GDR at 120 minutes is not statistically significant. Furthermore, in separate forward stepwise multiple regression analyses with the 60-, 90-, and 120-minute GDR, respectively, as the dependent variable, and the correlates significant for all 3 time points as independent variables, the same 2 variables (HDL cholesterol and Epi-A) remained significant in the final models. When 8 known correlates of insulin sensitivity[2,11,12,15] were entered, 2 of them (again HDL cholesterol and Epi-A) remained independent variables for the 60-, 90-, and 120-minute GDR, while BMI and Chol were significant in just 1 and 2 of the models, respectively.

There was a significant negative correlation between Hgb and the 60-minute GDR. We found this interesting, since we have previously found a negative association between hematocrit and whole blood viscosity (WBV) and insulin sensitivity in this population.[9,15] Thus, Hgb may, through its close relation to hematocrit, be associated with GDR. However, we cannot rule out that this is a chance finding, especially since the correlations to the 90- and 120-minute GDR are nonsignificant.

The lack of consensus concerning the optimal length of the glucose clamp is related to the difficulty of achieving steady-state glucose uptake.[7,8] While some investigators suggest that the 120-minute clamp is sufficient for estimating insulin sensitivity,[27,28] others[29] state that at least 180 minutes are required. However, the kinetics of insulin action differ between groups. The rate of onset of insulin action is reduced in obesity,[30] NIDDM,[31] and hypertension associated with obesity.[32] Data by Doberne et al[33] indicate that glucose utilisation, even in non-obese subjects with normal glucose tolerance, continues to increase progressively through 5 hours of hyperinsulinemia. On the other hand, Olsen et al[34] recently found that the glucose infusion rate did not change between 2 hours and 3 hours of insulin infusion in young, lean and healthy subjects.

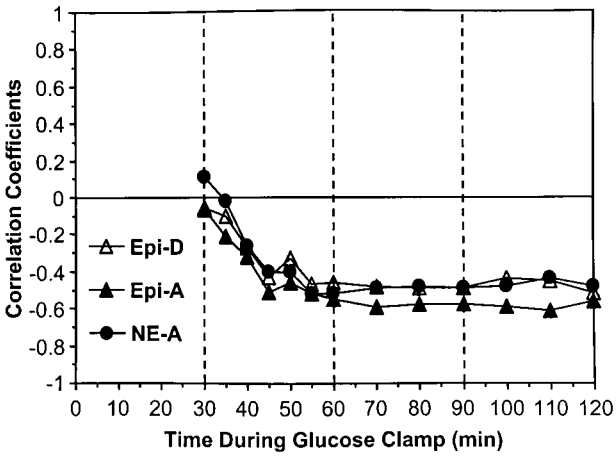


**Fig 4. Correlation coefficients between GDR at different time points and plasma catecholamine levels during MST. Markers indicate time points for calculation of GDR and the correlation coefficients. NE-A, indicates plasma norepinephrine 1 minute after announcement of MST; Epi-D, plasma epinephrine during announcement of MST; Epi-A, plasma epinephrine 1 minute after announcement of MST.**

The subjects in the present study had borderline hypertension but were otherwise healthy and, with the exception of 2 subjects, relatively lean (BMI $< 30$ kg/m$^2$). We propose that, in this type of subject, GDR calculated from 20-minute periods from 60 minutes onwards during the glucose clamp may be useful when studying the relationship between insulin sensitivity and other cardiovascular risk factors. In this context, possibly with the exception of *r* values between GDR and Chol and TG, the last 30 minutes of the 120-minute clamp added limited information to that obtained after 60 or 90 minutes. As we have not done this type of analysis on other populations (eg, elderly subjects or subjects with established hypertension and/or NIDDM) our statement is limited to this one.

A shorter version of the glucose clamp may have several advantages when compared to standard length clamps. Subjects may with time experience increasing discomfort from the procedure per se, leading to activation of the SNS.[21,35] Sympathetic activation has been shown to induce acute insulin resistance in the forearm,[36,37] and one may speculate that this could influence glucose clamp results in individuals with high SNS reactivity. Also, as discussed by Olsen et al,[34] the effect of insulin within the first hour of infusion is more relevant from a physiological point of view than the maximal effect after several hours of infusion. Further, a short clamp procedure may be more time-effective while also improving the willingness among subjects to take part in such studies. On the other hand, a main challenge when performing the glucose clamp is to maintain isoglycemia by adjusting the glucose infusion rate according to blood glucose levels. Therefore, shorter clamps require more caution when adjusting glucose infusion, and good validation, as investigators then have less time to correct for over- or underestimations.

Although not the primary objective of this study, we performed regression analyses in addition to correlation analyses. The results should be interpreted with caution, because of the small sample size and the relatively large number of candidate independent variables, among which many are strongly interrelated. In search for an easy way to predict insulin sensitivity, an interesting object for future studies might be to explore the possibility of deriving an algorithm using readily measured parameters such as BP, HR, lipid profiles, BMI and other anthropometrical parameters, and fasting plasma insulin. This is beyond the scope of this analysis, as the material is small and our aim is a simplified version of the hyperinsulinemic glucose clamp (ie, 60 to 90 minutes of steady, weight-adjusted insulin infusion and without tritiated glucose) for research purposes.

In conclusion, these data suggest that 60- to 90-minute hyperinsulinemic glucose clamps may provide the same information as 120-minute clamps regarding relationships between insulin sensitivity and various cardiovascular risk factors in young borderline hypertensive but otherwise healthy caucasian men.

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