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PET Detection of the Impact of Dobutamine on Myocardial Glucose Metabolism in Women with Type-1 Diabetic Mellitus

Pilar Herrero¹, Janet McGill², Donna S. Lesniak¹, Carmen S. Dence¹, Shalonda W. Scott¹, Zulfia Kisrieva-Ware¹, and Robert J. Gropler¹

¹Division of Radiological Sciences, Mallinckrodt Institute of Radiology, Washington University School of Medicine, 660 S. Euclid Ave, St. Louis, Missouri, 63110, USA

²Division of Endocrinology, Department of Internal Medicine Washington University School of Medicine, 660 S. Euclid Ave, St. Louis, Missouri, 63110, USA

Abstract

Objective—To determine in the hearts of women with type-1 diabetes mellitus (T1DM), if the fate of extracted glucose is altered and if so what is the impact of dobutamine on myocardial substrate metabolism.

Background—In experimental models of T1DM, myocardial glycolysis and glucose oxidation are reduced with the impairment becoming more pronounced with dobutamine. Whether similar changes in occur in humans with T1DM is unknown.

Methods—Myocardial perfusion, oxygen consumption, glucose and fatty acid metabolism were measured with PET in 19 women, 7 normal volunteers (NV) and 12 with T1DM. The NV and 6 T1DM (DM1) were studied under baseline metabolic conditions and 6 T1DM studied during hyperinsulinemic-euglycemic clamp (DM1-C) both at rest and during dobutamine.

Results—At rest, myocardial glucose uptake, glycolysis, glycogen storage and oxidation were reduced by similar levels in DM1 compared with NV ($P < .05$). During dobutamine, although myocardial glucose uptake was not different from DM1 at rest, fractional glycolysis was lower compared with NV or DM1-C and reflected a lower glucose oxidation rate ($P < .001$). Measurements of myocardial glucose metabolism at rest and during dobutamine were comparable between NV and DM1-C. During dobutamine, myocardial fatty acid uptake and oxidation increased in all 3 groups.

Conclusions—In women with T1DM, 1) myocardial glucose metabolism is impaired downstream from initial uptake, 2) these abnormalities become more pronounced with dobutamine and are paralleled by an increase in myocardial fatty acid metabolism, and 3) insulin restores glucose metabolism to levels observed in normal controls.

Keywords

Diabetes mellitus; metabolism; catecholamines; tomography

To whom correspondence should be addressed: Robert J. Gropler, MD, Box 8225, 510 South Kingshighway, St. Louis, MO 63110, Facsimile: (314)-747-3882, Phone: (314)-747-3878, Groplerr@mir.wustl.edu.

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Introduction

The metabolic phenotype of the diabetic heart is an over-dependence on fatty acid metabolism that is paralleled by a decline in glucose use, at least under euglycemic conditions (1-6). Results of studies in a wide range of experimental models of diabetes have documented that in addition to a decrease in glucose uptake there is a reduction in glycolysis and glucose oxidation (7-9). Results of studies in patients with either type-1 diabetes mellitus (T1DM) or type-2 diabetes mellitus have generally confirmed the increase in myocardial fatty acid utilization and oxidation and a decline in myocardial glucose uptake (1-3,10,11). However, whether the further metabolism of extracted glucose is reduced in humans with diabetes is unknown.

Moreover, much of what is known about the metabolic perturbations in patients with either T1DM or T2DM is limited to resting conditions. For example in humans with T1DM, atrial pacing results in an increase in myocardial glucose uptake without any change in fatty acid use. However, whether defects are present in glucose metabolism downstream from uptake is unknown (1). Furthermore, whether these metabolic perturbations are amenable to therapies such as supplemental insulin is unknown. Accordingly, in the current study we sought to answer 3 different questions. First, is the metabolism of extracted glucose by the heart reduced in patients with T1DM? Second, is the myocardial metabolic response to dobutamine, particularly as it related to glucose uptake and downstream metabolism, different between patients with T1DM and non-diabetics? Third, if differences in metabolism do exist, can they be reduced by the administration of insulin?

Methods

Study population

We studied 19 healthy women, 7 normal volunteers (NV) and 12 with T1DM. We studied only women because we recently reported that gender may impact myocardial substrate metabolism (12). Although type-2 diabetes mellitus is more prevalent, we purposefully chose to study only patients with T1DM to avoid the possible confounding effects of obesity and hypertension that often accompany type 2 diabetes (13,14). Non-diabetic women were identified based on clinical evaluation and a normal oral glucose tolerance test. Women were classified as T1DM based on the need for supplemental insulin the first year, a history of ketoacidosis, and a plasma C-peptide level of $<0.50\mu\text{mol/mL}$. No T1DM subject had active retinopathy, clinically significant autonomic neuropathy, or had a serum creatinine $>1.5\text{mg/dL}$. Sedentary women were chosen to minimize the possible confounding effects of variable levels in training-induced adaptations on myocardial substrate metabolism (15). All women were nonsmokers, normotensive, and without a family history of coronary artery disease had a normal physical exam, electrocardiogram, and rest/exercise echocardiogram. The study was approved by the Human Studies and the Radioactive Drug Research Committees at the Washington University School of Medicine. Written informed consent was obtained from all subjects before enrollment into the study.

Experimental procedure

All studies were performed on a conventional commercially available Tomograph (Siemens ECAT 962 HR+, Siemens Medical Systems, Iselin, New Jersey). All subjects were admitted overnight to the General Clinical Research Center at Washington University. Two 18- or 20-gauge catheters were placed into 2 different i.v. sites: 1 for infusion and one for blood sampling. At 6 PM the night before the study, both diabetic and non-diabetic subjects ingested a standard weight-adjusted meal. In the morning the NV ingested a second meal 2 hrs before starting the PET study. Six diabetic patients (DM1) were fasted until the following morning but overnight received an insulin drip at physiologic replacement doses (1-2 units/hr) and supplemental D5W

to maintain blood glucose levels of 5-7 mmol/L that was maintained until completion of the imaging study the next day. In this way NV and DM1 could be matched for their plasma insulin and glucose levels under resting conditions. The other 6 diabetic patients were started on a hyperinsulinemic-euglycemic clamp (DM1-C) 2 hours prior to the PET imaging session using standard methodology (16). All subjects were placed on telemetry and had blood pressures obtained routinely throughout the study. Rate-pressure product was calculated as systolic blood pressure*heart rate. All subjects were studied at 08:00 AM to avoid circadian variations in myocardial metabolism and function (17). Each subject underwent PET imaging on 2 separate days. On Day 1 the PET study was performed under the metabolic conditions described above at rest. On Day 2 the study was repeated under the same metabolic conditions as Day 1 but during the concomitant intravenous administration of dobutamine (10 µg/kg/min).

PET Image Acquisition

PET was used to measure MBF(mL/g/min), MVO₂ (µmol/g/min), glucose and fatty acid metabolism (nmol/g/min) using ¹⁵O-water; 1-¹¹C-acetate; 1-¹¹C-glucose and 1-¹¹C-palmitate, respectively, as reported previously (18-22). During the study, venous blood samples were obtained during each imaging portion of the study (i.e., during MBF, MVO₂, myocardial glucose and fatty acid metabolism imaging) to measure plasma substrates; glucose (µmol/min), fatty acids, lactate (nmol/mL) and insulin (µU/mL) levels and radiolabeled metabolites (19-22). The imaging protocol is summarized in Figure 1.

PET Image Analysis

Blood and myocardial PET time-activity curves were used in conjunction with well-established kinetic models to quantify MBF (mL/g/min), MVO₂ (µmol/g/min), myocardial fatty acid uptake (MFAU; nmol/g/min) and oxidation (MFAO; nmol/g/min) and overall myocardial glucose uptake (MGU; nmol/g/min (19-21). Recently, the glucose compartmental model we originally developed to measure myocardial glucose uptake has been optimized to permit measurements of the metabolic fate of myocardial glucose, including glycolysis, glucose oxidation and glycogen storage (22).

Measurement of Plasma Insulin and Substrates

Plasma insulin was measured by radioimmunoassay. Plasma glucose and lactate levels were measured using a commercially available glucose-lactate analyzer (YSI, Yellow springs, Ohio). The level of fatty acid in the plasma was determined by capillary gas chromatography and HPLC.

Statistical analysis

SAS software (SAS Institute) was used for the statistical analyses. Data are expressed as the mean values ± the SD. All group comparisons for continuous variables were done using two-way ANOVA for repeated measurements analyses with rest/dobutamine as the repeated factor (Dobutamine) and NV/DM1/ DM1-C as the grouping factor (Group). Post Hoc analyses were done only if P values < .05 were obtained for dobutamine, Group or their interaction (Dobutamine*Group).

Results

Clinical Characteristics

The clinical characteristics of the groups are shown in Table 1. The groups were well-matched for age, body mass index, plasma lipid levels and resting left ventricular function (P = NS). The DM1 and DM1-C groups had similar duration of disease and level of glycemic control.

Hemodynamics, MBF and MVO₂

Shown in Table 2 are values for the rate-pressure product, MBF and MVO₂. There were no differences among groups for values obtained either at rest or during dobutamine. As anticipated, during dobutamine there was a significant increase in RPP, MBF, and MVO₂ from rest conditions to in all 3 groups.

Plasma Insulin and Substrate Levels

Shown in Table 3 are the plasma insulin, glucose, fatty acid and lactate levels for the 3 groups. As anticipated by the intervention, plasma insulin levels were significantly higher in the DM1-C than in NV and DM1, both at rest ($P < .0005$) and during dobutamine ($P < .05$). On average plasma glucose levels were higher in DM1-C than DM1 and NV and higher in DM1 than NV both at rest and during dobutamine ($P < .05$). Glucose levels did not change with dobutamine in any of the 3 groups. Plasma fatty acid levels were higher in DM1 when compared with NV and DM1-C, both at rest ($P < .005$) and during dobutamine ($P < .01$). On average, fatty acid levels increased in all groups with dobutamine ($P < .01$). At rest, plasma lactate levels were highest in DM1-C and lowest in DM1 ($P < .01$). On average, plasma lactate levels increased with dobutamine ($P < .05$) but no differences were noted among the 3 groups.

Myocardial Fatty Acid Metabolism

Shown in Figure 2 are the measurements of myocardial fatty acid metabolism in the 3 groups. MFAU (nmol/g/min) at rest was highest in DM1 (102 ± 42) followed by NV (60 ± 0.21) and lowest in the DM1-C (17 ± 9), $P < .05$. During dobutamine, a similar pattern was found with DM1 having the highest MFAU (213 ± 78) followed by NV (142 ± 92) with the lowest MFAU in DM1-C (61 ± 49), $P < .05$ vs. DM1. Of note, MFAU significantly increased with dobutamine in all 3 groups ($P < .001$).

MFAO (nmol/g/min) at rest was highest in DM1 (99 ± 42 , $P < .01$) when compared with NV (43 ± 24), and DM1-C (18 ± 30). During dobutamine, MFAO was similar between DM1 (191 ± 75) and NV (133 ± 79) but lower in DM1-C (44 ± 46 , $P < .05$). There was significant increase in MFAO with dobutamine in all groups ($P < .0005$).

At rest, the fraction of extracted fatty acid that was oxidized (%MFAO) was similar between NV ($72 \pm 26\%$) and DM1 ($97 \pm 2\%$) but significantly lower in DM1-C ($33 \pm 30\%$, $P < .005$). However, during dobutamine no differences were noted among groups, NV ($93 \pm 6\%$), DM1 ($90 \pm 10\%$), and DM1-C ($79 \pm 14\%$). Of note, there was significant increase in %MFAO with dobutamine compared with rest conditions in the DM1-C ($P < .05$). However, no increases were observed in DM1 or NV.

Myocardial Glucose Metabolism

Shown in Figure 3 are MGU, glycolysis and glycogen synthesis (all in nmol/g/min) for the 3 groups, both at rest and during dobutamine. At rest, MGU levels were comparable between the NV (493 ± 188) and DM1-C (693 ± 353) groups but significantly lower in the DM1 group (186 ± 76), $P < .05$. A similar pattern was observed during dobutamine with MGU values being comparable between the NV (478 ± 205) and DM1-C (820 ± 536) groups but significantly lower in the DM1 group (154 ± 105), $P < .05$. Of note, MGU did not increase with dobutamine in any of the groups. Under resting conditions, glycolysis and glycogen synthesis (nmol/g/min) were lower in DM1 (76 ± 47 and 110 ± 41) compared with either NV (292 ± 143 and 201 ± 82) or DM1-C (348 ± 259 and 345 ± 133), $P < .05$. These differences were commensurate with differences in MGU among the groups. During dobutamine, rates of glycolysis were lower in DM1 (61 ± 75), compared with either NV (320 ± 175) or DM1-C (562 ± 525), $P < .05$. Rates of glycogen synthesis were also lower in DM1 (93 ± 51), compared with DM1-C (252 ± 91 ;

$P < .05$) but similar to NV (148 ± 36). However, the glycolytic fraction (relative to total glucose uptake) was significantly lower in the DM1 group ($30 \pm 23\%$) compared with either the NV ($59 \pm 17\%$) or DM1-C ($62 \pm 15\%$) groups, $P < .05$. Paralleling this pattern was the higher glycogen synthesis fraction in the DM group ($70 \pm 23\%$) compared with either the NV ($41 \pm 17\%$) or DM1-C ($38 \pm 15\%$) groups, $P < .05$.

Shown in Figure 4 are the rates of the glucose oxidation (in nmol/g/min) for the 3 groups. At rest, rates of glucose oxidation was lower in DM1 (53 ± 32) compared with either NV (226 ± 140) or DM1-C (312 ± 224), $P < .05$. These differences paralleled the differences in MGU among the groups. Similar patterns were observed during dobutamine, with rates of glucose oxidation being lower in DM1 (38 ± 47) compared with either NV (250 ± 160) or DM1-C (451 ± 423), $P < .05$. Of note, during dobutamine, fractional glucose oxidation was lower in DM1 ($18 \pm 12\%$) when compared with either the NV ($48 \pm 0.12\%$) or DM1-C ($50 \pm 12\%$) ($P < .001$), suggesting an greater impairment in glucose oxidation relative to glucose uptake in DM1.

Discussion

The novel findings of the current study are that in female patients with T1DM: 1) Under resting conditions, myocardial glycolysis, glucose oxidation and glycogen synthesis are reduced in proportion to the decline in glucose uptake; 2) During dobutamine, glycolysis and glucose oxidation are reduced relative to uptake resulting in a greater fraction of extracted glucose undergoing glycogen synthesis whereas there is an increase in myocardial fatty acid uptake and oxidation and; 3) The impairment in glucose uptake, glycolysis and glucose oxidation both at rest and during dobutamine can be restored by the administration of high dose insulin.

Myocardial g metabolism-resting conditions

Consistent with the results of prior studies in experimental models of T1DM and patients with the disease, myocardial fatty acid metabolism was increased in the DM1 compared with NV most likely due to the increase in plasma fatty acid levels. The findings of reduced myocardial glycogen synthesis, glycolytic, and glucose oxidation rates commensurate with the decline in glucose uptake confirms that the perturbations in myocardial glucose metabolism in the human diabetic heart are similar to that of observed in experimental models of the disease (4,23-26). Likely components of the glucose metabolic pathway targeted by diabetes include a decline in myocardial glucose transporter function (e.g., Glucose transporters 1 and 4), and a decline in activity of hexokinase and 6-phosphofructo-2-kinase (glycolysis), and pyruvate dehydrogenase complex (oxidation) (4,23-26).

In the current study, administration of pharmacological doses of insulin reversed most of these abnormalities. The decrease in MFAU, MFAO and %MFAO (Figure 2) likely reflects the combined effects of reduced fatty acid delivery to the myocardium (due to decreased peripheral lipolysis) and increased myocardial malonyl-CoA levels. Conversely, the increase in MGU likely reflects the increased translocation of GLUT-4 to the sarcolemma (27,28). Increases in glycolysis and glucose oxidation are consistent the effects of reduced peroxisome proliferator-activated receptor α activation leading to reduced pyruvate dehydrogenase kinase 4 activity as well direct effects of insulin on glycolytic (i.e., 6-phosphofructo-2-kinase) and oxidative components (pyruvate dehydrogenase complex) (4,9). Similarly, the increase in glycogen synthesis likely reflected the stimulatory effects of reduced cellular fatty acids and perhaps insulin on glycogen synthase activity.

Myocardial substrate metabolism – dobutamine

MFAU and MFAO increased during dobutamine to similar levels and by equivalent amounts in T1DM patients when compared with non-diabetics (Figure 2). This increase in fatty acid

metabolism appeared to be driven primarily by the increase in plasma fatty acid levels, likely reflecting the lipolytic effects of beta-adrenergic stimulation in peripheral adipose tissue by hormone-sensitive lipase (29). In isolated perfused non-diabetic hearts, catecholamine stimulation results in an increase in MGU, glycolysis and oxidation (30). In non-diabetic humans studied under fasting conditions, dobutamine infusion resulted in a significant increase in plasma insulin levels which in turn was associated with an increase in myocardial glucose uptake (31). The lack of an increase in MGU in the current study, compared to the increase in MGU reported previously, may simply reflect differences in the experimental conditions. For example, in the current study, normal subjects were studied in the fed state which leads to an increase plasma insulin levels. As a consequence, a significant increase in plasma insulin levels with dobutamine was not observed and may have contributed to the lack of increase in myocardial glucose uptake.

With dobutamine, the metabolic fate of extracted glucose differs between NV and DM1 groups. The fraction of glucose metabolism representing glycolysis was significantly lower in the DM1 group and moreover, tended to be lower when compared with resting conditions. This reduction in glycolysis reflected primarily a reduction in glucose oxidation. As a consequence, the fraction of extracted glucose undergoing glycogen synthesis was increased. Given that plasma fatty acids increased with dobutamine, these results are consistent with those of prior studies in various experimental models of T1DM demonstrating that in the setting of increased myocardial fatty acid delivery, the impairment in myocardial glucose oxidation appears to be greater than the impairment in glycolysis which in turn is greater than the impairment in glucose uptake leading to a relative increase in glycogen stores (4,23-26). The impairment in glucose oxidation with dobutamine is consistent with a defect in pyruvate oxidation (23).

At pharmacological doses of insulin (DM1-C) myocardial uptake of glucose did not increase during dobutamine (Figure 3). Yet, an increase in MFAU and MFAO was seen, although at lower levels than that observed under baseline metabolic conditions (DM1) (Figure 2). These data suggest that the lipolytic effects of dobutamine can overcome the lipogenesis effects of insulin to increase plasma fatty acid delivered to the myocardium and thus stimulate myocardial fatty acid metabolism. In addition, it appears the administration of insulin was also able to overcome the reduction in glucose oxidation during dobutamine.

Limitations

It is unlikely that the differences in myocardial substrate metabolism were attributable solely to the differences in the plasma substrate and insulin environment between the 3 groups. For example, at rest, DM1 patients exhibited lower levels of glucose oxidation compared with NV despite, on average, higher (but not significant) plasma insulin levels. Moreover, at rest, even though DM1-C exhibited ~8× higher plasma insulin levels than NV, the level of glucose oxidation was similar between the groups further emphasizing the impairment myocardial carbohydrate oxidation in T1DM. The results of the current study can only be applied to females with and without T1DM. Further studies will be required to determine the applicability of these observations in males and in patients with T2DM. The compartmental modeling method used to estimate glycogen synthesis is based on the fraction of tracer that enters slow turn over pools and thus remains within tissue. There are other glucose intermediates such as those of the pentophosphate pathway that may also show a slow turnover pool pattern. Thus it is possible that the fraction of extracted glucose that entered glycogen synthesis was overestimated in our study.

Conclusions

The results of the current study further complete the picture of the myocardial metabolic phenotype in humans with T1DM. More specifically, reductions in myocardial glucose use

extend beyond initial uptake and include various aspects of downstream glucose metabolism that become more pronounced with dobutamine. In contrast, the over-dependence by the heart on fatty acid metabolism is increased during dobutamine. Further study will be required to determine the extent to which these metabolic alterations may contribute to the cardiac abnormalities typically observed in diabetes mellitus and may be targets for metabolic therapies in this disease.

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PET Imaging Protocol

Figure 1.

Imaging protocol. MBF: myocardial blood flow, MVO₂: Myocardial oxygen consumption; MGM: Myocardial glucose metabolism, MFAM: myocardial FFA metabolism; Substrates: Glucose, Fatty Acids and Lactate, ^{11}C -metabolites: $^{11}\text{CO}_2$ and ^{11}C -lactate.

Figure 2.

Measurements of myocardial fatty uptake and oxidation. MFAU: Myocardial fatty acid uptake; MFAO: Myocardial fatty acid oxidation; MFAO (%): Fraction of extracted fatty acid that undergoes β -oxidation. **MFAU**: Rest vs. Dobutamine, $P < .001$; $\#P < .05$ vs. NV(Rest); $*P < .05$ vs. NV(Rest) and DM1(Rest); $**P < .05$ vs. DM1(Dobutamine); **MFAO**: Rest vs. Dobutamine, $P < .0005$; $*P < .01$ vs. NV(Rest) and DM1-C (Rest); $\#P < .05$ vs. NV (Dobutamine) and DM1(Dobutamine); **MFAO (%)**: Rest vs. Dobutamine, $P = \text{NS}$, $*P < .005$ vs. NV(Rest) and DM1(Rest); $\#P < .05$ vs. DM1-C(Rest).

Figure 3.

Measurements of myocardial glucose uptake (MGU), glycolysis and glycogen synthesis.

MGU: Rest vs. Dobutamine, $P = \text{NS}$, $**P < .05$ vs. NV (Rest and Dobutamine) and DM1-C (rest and Dobutamine); **Myocardial glycolysis:** Rest vs. Dobutamine, $P = 0.09$, $*P < .05$ vs. NV (Rest and Dobutamine) and DM1-C (Rest and Dobutamine); **Myocardial glycogen synthesis:** Rest vs. Dobutamine, $P = .07$, $**P < .05$ vs. NV (Rest) and DM1-C (Rest); $\#P < .05$ vs. DM1-C (Rest);

Figure 4.

Measurements of myocardial glucose oxidation. **Glucose oxidation:** Rest vs. Dobutamine, P = NS; *P < .05 vs. NV (Rest and Dobutamine) and DM1-C (Rest and Dobutamine).

Clinical Characteristics**Table 1**

	NV	DM1	DM1-C
Subjects (women)	7	6	6
Age (yrs)	27 ± 4	33 ± 9	34 ± 11
BMI (kg/m ²)	23 ± 3	25 ± 3	27 ± 4
TG (mg/dL)	67 ± 18	72 ± 53	58 ± 29
LVEF (%)	67 ± 4	65 ± 6	65 ± 6
DM duration (yrs)		21 ± 10	17 ± 10
HbA _{1C} (%)		9.1 ± 2.4	9.3 ± 2.9

DM: Diabetes Mellitus; BMI: Body mass index; TG: triglycerides; LVEF: Left ventricular ejection fraction; Hb: Hemoglobin

Table 2

Rate Pressure Product, MBF and MVO₂

	NV		DMI		DMI-C		P-values
	Rest	Dob	Rest	Dob	Rest	Dob	
RPP (BMP • mmHg)	6943 ± 1230	12275 ± 2013 *	8901 ± 2138	14194 ± 7082 *	9221 ± 2100	21406 ± 12567 *	Dob: < .0005 Group: NS
MBF (mL/g/m)	1.07 ± 0.13	2.08 ± 0.37 *	1.20 ± 0.27	2.83 ± 0.74 *	1.08 ± 0.17	2.73 ± 0.69 *	Dob: < .0001 Group: NS
MVO ₂ (μmol/g/m)	4.91 ± 0.94	12.7 ± 1.64 *	6.10 ± 1.61	18.6 ± 11.8 *	7.34 ± 3.28	14.74 ± 5.82 *	Dob: < .0001 Group: NS

Dob: Dobutamine; RPP: Rate pressure product; MBF: Myocardial blood flow; MVO₂: Myocardial Oxygen consumption;

* P < .01 vs. Rest

Table 3

Plasma Insulin and Substrates

	NV		DMI		DMI-C		P-values
	Rest	Dob	Rest	Dob	Rest	Dob	
Insulin (μ U/mL)	12.6 $\pm$ 10.3	33.4 $\pm$ 33.1	25.0 $\pm$ 40.7	62.9 $\pm$ 86.0	95.5 $\pm$ 21.3*	105.0 $\pm$ 31.7**	Dob: P = .07 Group: P < .005
Glucose (μ mol/mL)	4.96 $\pm$ 0.73	5.16 $\pm$ 0.46		$\ddagger$		$\ddagger, \ddagger\ddagger$	
FFA (nmol/mL)	227 $\pm$ 137	426 $\pm$ 162*	582 $\pm$ 284#	1044 $\pm$ 475##	82 $\pm$ 87	357 $\pm$ 372	Dob: P = NS Group: P < .005 Dob: P < .01 Group: P < .0001
Lactate (nmol/mL)	866 $\pm$ 141	996 $\pm$ 256	564 $\pm$ 119\$	989 $\pm$ 425	1128 $\pm$ 144\$\$	1177 $\pm$ 319	Dob: P < .05 Group: P < .05

NV: Normal volunteers; DMI: Type 1 DM controls; Type 1 DM studied during hyperinsulinemic-euglycemic clamp; Dob: Dobutamine,

* Insulin: P < .0005 vs. NV(Rest) and DMI(Rest),

** Insulin: P < .05 vs. NV(Dob) and DMI(Dob)

$\ddagger$ Glucose: P < .05 vs. NV,

$\ddagger\ddagger$ Glucose: P < .05 vs. DMI,

Free Fatty Acids (FFA): P < .005 vs. NV(Rest) and DMI-C(Rest),

Free Fatty Acids (FFA): P < .01 vs. NV(Dob) and DMI-C(Dob)

\$ Lactate: P < .005 vs. NV(Rest),

\$\$ Lactate: P < .01 vs. NV(Rest) and DMI (Rest)