SUPPLEMENTARY DATA

1. Supplemental Tables

2. Supplemental Figures

Overnight intravenous insulin infusion protocol for type 1 diabetes participants

SUPPLEMENTARY DATA

Supplementary Table S1.
Inclusion and Exclusion Criteria for Study Participants

Inclusion Criteria		Exclusion Criteria	
All subjects			
BMI	Age 13-18: 15%-85% percentile Age 18-51: 19-28 kg/m^2	Severe hypoglycemia	≥1 episode in the past 3 months or diagnosis of hypoglycemia unawareness
		Diabetes comorbidites	- ≥1 trip to emergency room for poor glucose control in the past 6 months - New York Heart Association Class II-IV cardiac functional status - SBP >140 mmHg and DBP > 100 mmHg - Fasting triglycerides > 400 mg/dL - Liver transaminases > 2 times upper limit of normal - Renal transplantation or serum creatinine > 1.5 mg/dL
		Medications	Any systemic glucocorticoid, any antipsychotic, atenolol, metoprolol, propranolol, niacin, any thiazide diuretic, any OCP with > 35 mcg ethinyl estradiol, growth hormone, any immunosuppressant, any antihypertensive, any antihyperlipidemic
		Other	Pregnancy, Tanner stage < 5, peri- and post-menopausal women
T1DM subjects			
Age	13-51 years	Medications	Any diabetes medication except insulin
T1DM duration	1-20 years	C-peptide	> 0.7 ng/mL (fasting)
HbA1c	5.9-7.5% (41-58 mmol/mol)		
GCK-MODY subjects			
Age	13-51 years		
HbA1c	5.9-7.5% (41-58 mmol/mol)		
Positive GCK sequencing			
Control subjects			
Age	18-51 years		
HbA1c	<5.5% (<37 mmol/mol)		

SUPPLEMENTARY DATA

Supplementary Table S2.

Baseline values for key factors affecting insulin sensitivity between cohorts. Data were collected during the screening visit after an overnight fast. GCK-MODY = Glucokinase Maturity-onset diabetes of the young (also known as MODY2). T1DM = type 1 diabetes. RH-PAT = reactive hyperemia-peripheral artery tonometry. Table reports mean values ± standard deviation.

Parameter	Control (n=10)	GCK-MODY (n=10)	T1DM (n=10)
HbA1c (%)	4.8 ± 0.4	6.2 ± 0.3	6.6 ± 0.5
HbA1c (mmol/mol)	29 ± 4.4	44 ± 3.3	49 ± 5.5
Age (years)	25.3 ± 3.0	24.8 ± 9.1	26.3 ± 7.8
BMI (kg/m^2)	22.5 ± 2.6	21.5 ± 1.9	23.0 ± 1.5
Body fat (%)	29.1 ± 9.8	30.7 ± 6.8	26.7 ± 5.8
VO2 max (mL/kg/min)	38.1 ± 5.3	39.9 ± 9.3	38.8 ± 5.7
Resting energy expenditure (kcal/day)	1531 ± 337	1415 ± 207	1639 ± 235
Systolic blood pressure (mmHg)	114 ± 10	116 ± 13	116 ± 10
RH-PAT score	2.03 ± 0.64	2.16 ± 0.63	1.99 ± 0.41
Triglycerides (mg/dL)	65 ± 26	65 ± 39	73 ± 24
HDL (mg/dL)	56 ± 9	55 ± 10	64 ± 11
LDL (mg/dL)	101 ± 26	101 ± 23	102 ± 30
Total cholesterol (mg/dL)	170 ± 36	168 ± 33	179 ± 39

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Supplementary Table S3.
Glucokinase mutations in the GCK-MODY participants

Participant Number	Nucleotide change	Protein Effect	Reference
1	c.659G>A	p.Cys220Tyr	(1-3)
2	c.215G>A	p.Gly72Glu	novel
3	c.1174C>T	p.Arg392Cys	(4)
4	c.556C>G	p.Arg186Gly	(5)
5	c.787T>C	p.Ser263Pro	(6)
6	c.572G>A	p.Arg191Gln	(7)
7	c.572G>A	p.Arg191Gln	(7)
8	c.619G>T	p. Val207Leu	novel
9	c.661G>A	p. Glu221Lys	(8)
10	c.863+1G>A	*	(9)

* c.863+1 G>A represents a nucleotide substitution in the canonical donor splice site of intron 7 that would be predicted to cause skipping of the exon 7 coding sequence and significant disruption to the protein sequence.

SUPPLEMENTARY DATA

Supplementary Table S4.

Characteristics of insulin regimens for type 1 diabetes participants (n=10). MDI = participants taking multiple daily injections, CSII = participants using continuous subcutaneous insulin therapy (a.k.a. insulin pump). Data presented as means ± SD.

Weight (kg)	72.0 ± 12.5
Prandial insulin dose (units/kg/day)	0.38 ± 0.19
Basal insulin dose (units/kg/day)	0.33 ± 0.10
Total daily dose (units/kg/day)	0.71 ± 0.25
Breakfast insulin to carbohydrate ratio	1 : 8.3 ± 2.7
Lunch insulin to carbohydrate ratio	1 : 8.2 ± 2.3
Supper insulin to carbohydrate ratio	1 : 9.0 ± 2.2
Insulin sensitivity factor (mg/kg/unit)	42.7 ± 12.5
MDI / CSII	3 / 7

SUPPLEMENTARY DATA

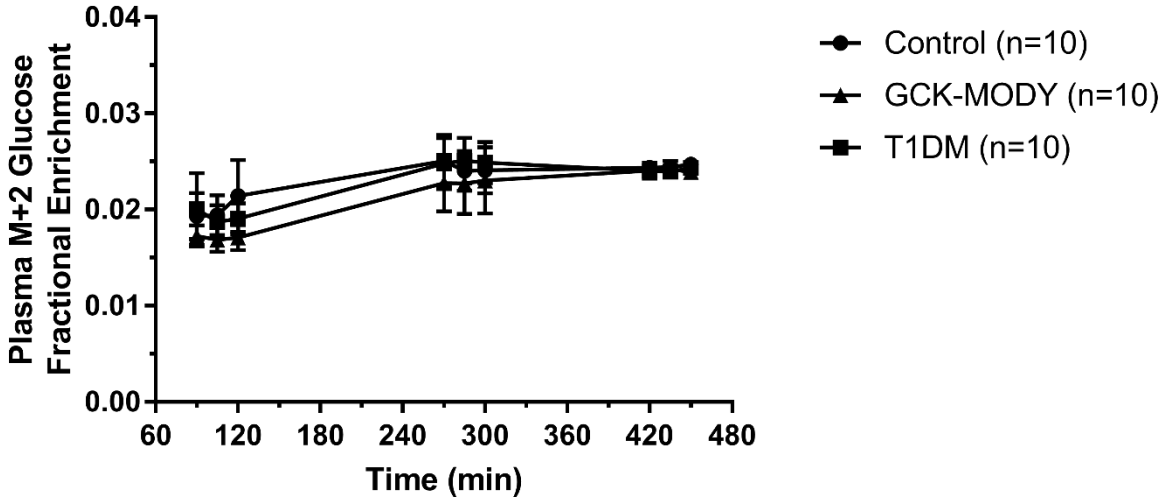
Supplementary Table S5.
Multivariate linear regression models testing which independent variables were most associated with muscle insulin sensitivity.

Linear regression models	Standardized coefficient for basal insulin concentration, β_1 (p-value)	Standardized coefficient for HbA1c, β_2 (p-value)	Standardized β_3 coefficient (p-value)	Model R^2
Adjusted for basal insulin (β_1) and HbA1c (β_2)	-0.58 (p=0.003)	-0.030 (p=0.87)	--	0.356
Adjusted for basal insulin (β_1), HbA1c (β_2), and BMI (β_3)	-0.56 (p=0.003)	-0.065 (p=0.72)	0.18 (p=0.25)	0.391
Adjusted for basal insulin (β_1), HbA1c (β_2), and REE (β_3)	-0.55 (p=0.009)	-0.087 (p=0.65)	0.061 (0.72)	0.378
Adjusted for basal insulin (β_1), HbA1c (β_2), and age (β_3)	-0.59 (p=0.003)	-0.038 (p=0.83)	-0.10 (p=0.53)	0.369
Adjusted for basal insulin (β_1), HbA1c (β_2), and percent fat mass (β_3)	-0.57 (p=0.005)	-0.036 (p=0.85)	0.031 (p=0.85)	0.359
Adjusted for basal insulin (β_1), HbA1c (β_2), and waist-to-hip ratio (β_3)	-0.58 (p=0.006)	-0.079 (p=0.70)	-0.28 (p=0.13)	0.455
Adjusted for basal insulin (β_1), HbA1c (β_2), and triglycerides (β_3)	-0.58 (p=0.003)	-0.034 (p=0.85)	0.026 (p=0.87)	0.359
Adjusted for basal insulin (β_1), HbA1c (β_2), and LDL (β_3)	-0.59 (p=0.003)	-0.025 (p=0.89)	-0.053 (p=0.74)	0.361
Adjusted for basal insulin (β_1), HbA1c (β_2), and HDL (β_3)	-0.59 (p=0.003)	-0.041 (p=0.82)	-0.058 (p=0.73)	0.362
Adjusted for basal insulin (β_1), HbA1c (β_2), and $VO2_{max}$ (β_3)	-0.59 (p=0.003)	-0.031 (p=0.86)	0.085 (p=0.59)	0.366
Adjusted for basal insulin (β_1), HbA1c (β_2), and basal NEFA (β_3)	-0.62 (p=0.007)	-0.02 (p=0.902)	-0.05 (p=0.778)	0.358

SUPPLEMENTARY DATA

Supplementary Figure S1.

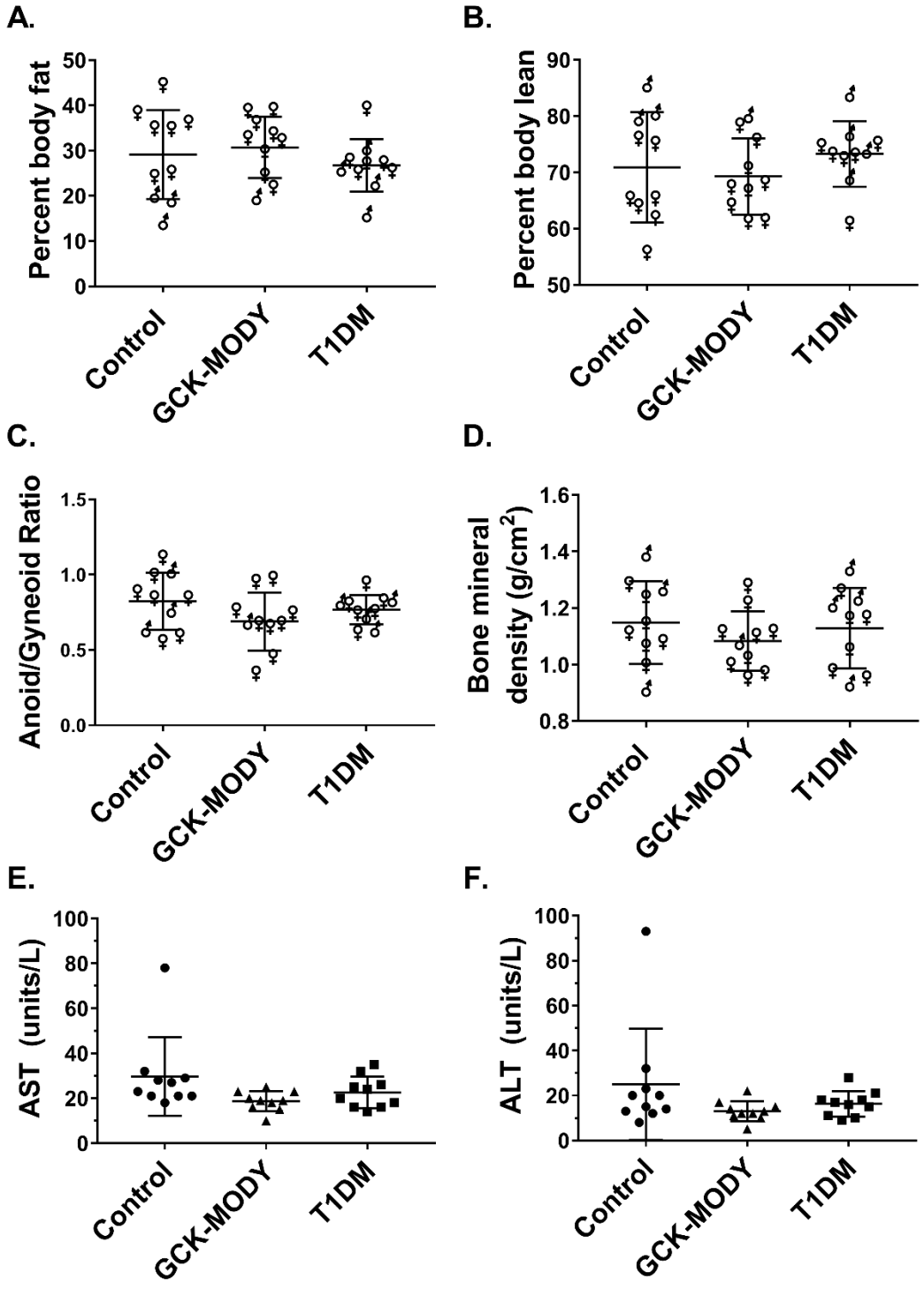
Fractional enrichment of the M+2 glucose isotope in plasma. Data depict means and SD.



SUPPLEMENTARY DATA

Supplementary Figure S2.

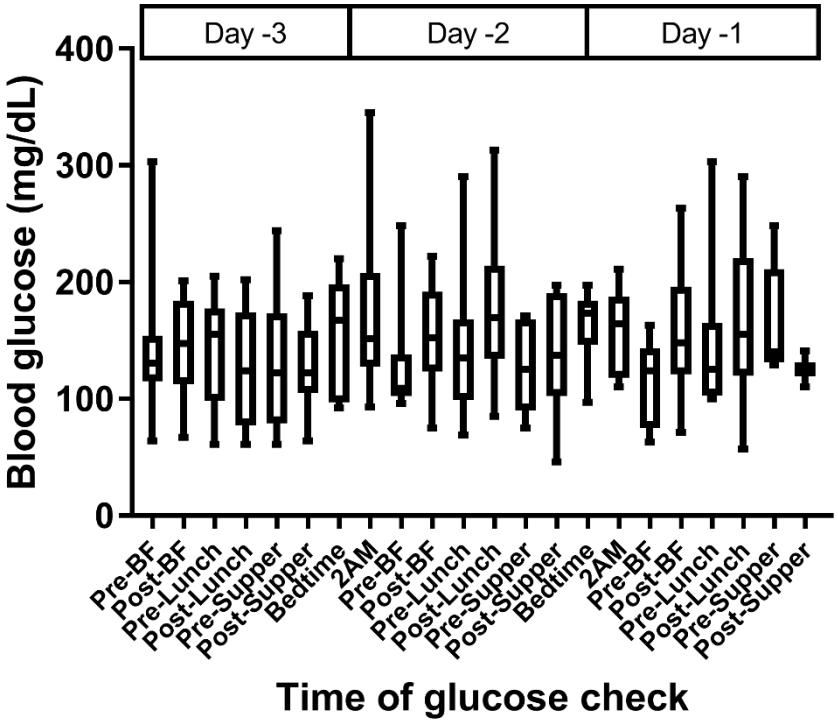
A) Percent body fat, B) percent lean body mass, C) anoid/gyneoid ratio, D) bone mineral density, E) blood aspartate aminotransferase (AST) concentrations, and F) blood alanine aminotransferase (ALT) concentrations for control, glucokinase maturity-onset diabetes of the young (GCK-MODY), and type 1 diabetes (T1DM) groups. Body composition parameters determined by dual-energy X-ray absorptiometry. Figures depict mean values and standard deviation.



SUPPLEMENTARY DATA

Supplementary Figure S3.

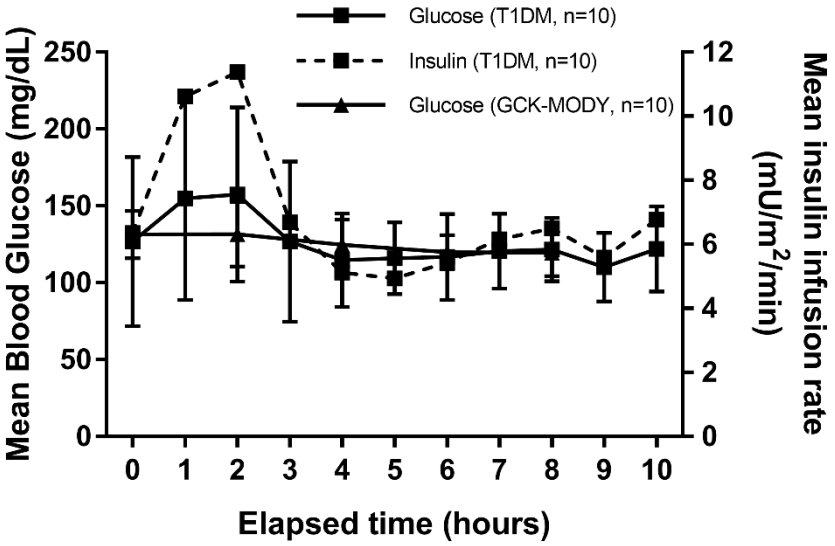
Box and whiskers plot depicting type 1 diabetes participants' self-monitored blood glucose in the three days prior to the clamp study. Horizontal line indicates median glucose, box represents the 25%-75% intra-quartile range, and vertical line represents minimum and maximum self-monitored blood glucose.



SUPPLEMENTARY DATA

Supplementary Figure S4.

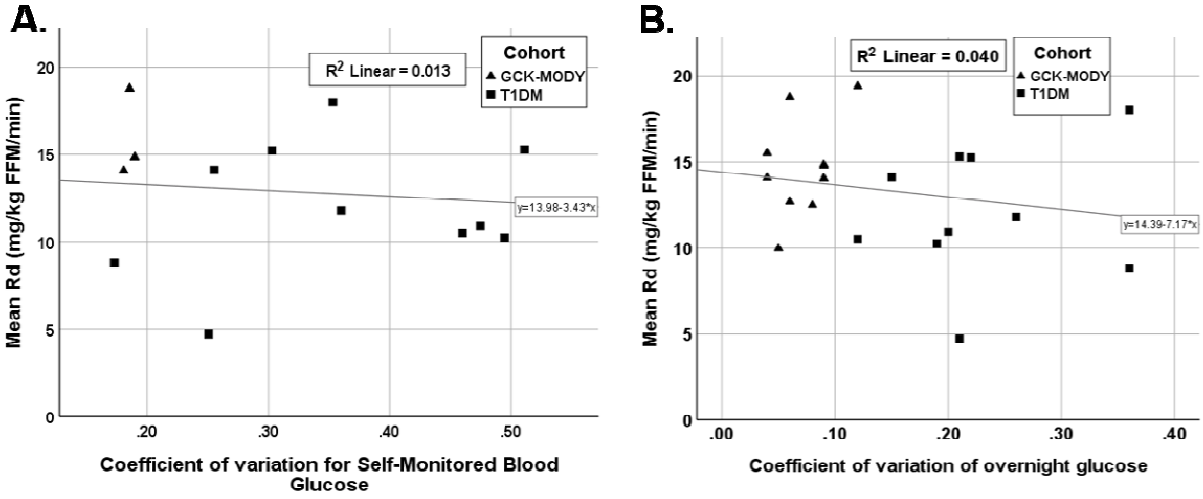
Plasma glucose concentrations (means and SD) and insulin infusion rates (means) during the overnight insulin infusion for type 1 diabetes and GCK-MODY participants. The participants began the overnight insulin infusion at 22:00 prior to the clamp study.



SUPPLEMENTARY DATA

Supplementary Figure S5.

Bivariate analyses of the effect of: A) coefficient of variation of self-monitored blood glucose (SMBG) on R_d during period 2 of the hyperinsulinemic euglycemic clamp, B) coefficient of variation of serial blood glucose readings overnight before the clamp on period 2 R_d. Corresponding Pearson product-moment correlation coefficients and two-tailed p-values are: A) r = -0.114 (p = 0.71), B) r = - 0.199 (p = 0.40).



SUPPLEMENTARY DATA

Overnight IV Insulin Infusion Protocol for Type 1 Diabetes Participants

Overnight Insulin Infusion Protocol

The following insulin infusion protocol is intended for use in T1DM and GCK-MODY participants in the setting of the overnight insulin infusion prior to hyperinsulinemic clamp studies. Target blood glucose is **90-119 mg/dL**.

Initiating the Insulin Infusion

1. START TIME: the overnight infusion should begin at 2200 the night before the clamp study unless directed otherwise
2. TRANSITIONING FROM HOME INSULIN REGIMEN: GCK-MODY participants in this study will not be taking insulin at home. T1DM participants will either use a basal insulin analog that is usually given every 24 hours or they will be on an insulin pump. Instructions for coming off the home insulin regimen is as follows:
    a. BASAL INSULIN: the study team will have instructed participants taking a basal insulin analog to transition to nightly dosing. On the night prior to the study, this basal insulin dose will simply be withheld and the IV insulin infusion will be started at 2200.
    b. INSULIN PUMP: those T1DM using an insulin pump should be asked to suspend and disconnect their insulin pump 15-20 minutes before starting the overnight insulin infusion (i.e. at 2140-2145).
3. PREPARATION FOR INFUSION: insulin will be dispensed as 50 units diluted in 50 mL of 0.9% NaCl.
    a. PRIMING: flush 10 mL of insulin infusion through all IV tubing before insulin infusion begins (to saturate insulin binding sites in the tubing).
    b. CARRIER FLUID: 0.9% NaCl fluid at 20 mL/hr can be run with the IV insulin infusion to keep the vein open.
    c. HYPOGLYCEMIA PREVENTION: 20% dextrose solution should be hung and ready in case of hypoglycemia. Glucagon should be within easy reach.
4. INITIAL IV INSULIN INFUSION RATE: initial infusion rate will be determined based on whether the participant is taking a basal insulin analog, is on an insulin pump, or has GCK-MODY. Please record the insulin infusion rate in the space provided on page 1 of the Task Flowsheet.
    a. BASAL INSULIN: divide the participants total daily basal insulin dose by 24 and *round down* to the nearest 0.25 unit/hr.
        Examples: 1) basal insulin dose = Lantus 30 units SQ q HS
        initial IV insulin infusion rate = 30 ÷ 24 = <u>1.25 unit/hr</u>
        2) basal insulin dose = Levemir 8 units SQ q 12 hours
        total daily basal insulin dose = 16 units
        initial IV insulin infusion rate = 16 ÷ 24 = 0.6667 = <u>0.5 unit/hr</u>
    b. INSULIN PUMP: start the IV insulin infusion at a rate equal to the home insulin pump basal rate.
        Examples: 1) home insulin pump basal rate = Humalog 0.8 units/hr
        Initial IV insulin infusion rate = 0.75 unit/hr
    c. GCK-MODY: the PI will provide the initial IV insulin infusion rate.

Blood glucose (BG) Monitoring

1. Check BG hourly on the hour and record BG and insulin infusion rate on the task flowsheet on the designated line.

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Changing the Insulin Infusion Rate

With each subsequent BG check, change the insulin infusion rate as follows:

IF BG < 55 mg/dL:

STOP INSULIN INFUSION - Bolus 100 mL of D20 (20 gm of glucose) immediately

-Contact PI
-Check BG q 15 minutes
-When BG is ≥ 90 mg/dL, wait 1 hour, recheck BG. If still ≥ 90 mg/dL, restart insulin infusion at 50% of the most recent rate

IF BG 55-69 mg/dL:

STOP INSULIN INFUSION - Contact PI immediately

-Prepare to give D20 at approximately 3.0 mg/kg/min after discussing with PI.
-Check BG q 15 minutes
-When BG is ≥ 90 mg/dL, wait 1 hour, recheck BG. If still ≥ 90 mg/dL, restart insulin infusion at 75% of the most recent rate

IF BG ≥ 70 mg/dL:

STEP 1: Determine the CURRENT BG LEVEL. This identifies a COLUMN in the table:

BG 70-89 mg/dL	BG 90-119 mg/dL	BG 120-179 mg/dL	BG ≥ 180 mg/dL

STEP 2: Determine the RATE OF CHANGE from the prior BG level. This identifies a CELL in the table. Then move to the right for **INSTRUCTIONS**. *(Note: if the last BG was measured less than an hour before the current BG, calculate the hourly rate of change. Example: if the BG at 0200 was 85 mg/dL and an additional BG is checked early at 0230 was 70 mg/dL, the total change over 30 minutes is -15 mg/dL; however, the hourly rate of change is -15 mg/dL x 2 = -30 mg/dL).*

BG 70-89 mg/dL	BG 90-119 mg/dL	BG 120-179 mg/dL	BG ≥ 180 mg/dL	INSTRUCTIONS*
		BG ↑ by > 40 mg/dL/hr	BG ↑	↑ INFUSION by "2Δ"
	BG ↑ by > 20 mg/dL/hr	BG ↑ by 1-40 mg/dL/hr OR BG UNCHANGED	BG UNCHANGED OR BG ↓ by 1-40 mg/dL/hr	↑ INFUSION by "Δ"
BG ↑	BG ↑ by 1-20 mg/dL/hr, BG UNCHANGED, OR BG ↓ by 1-20 mg/dL/hr	BG ↓ by 1-40 mg/dL/hr	BG ↓ by 41-80 mg/dL/hr	NO INFUSION CHANGE

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BG unchanged OR BG↓ by 1-20 mg/dL/hr	BG ↓ by 21-40 mg/dL/hr	BG ↓ by 41-80 mg/dL/hr	BG ↓ by 81-120 mg/dL/hr	↓ INFUSION by "Δ"
BG ↓ by > 20 mg/dL/hr see below†	BG ↓ by > 40 mg/dL/hr	BG ↓ by > 80 mg/dL/hr	BG ↓ by > 120 mg/dL/hr	HOLD x 30 min, then ↓ INFUSION by "2Δ"

***CHANGES IN INSULIN INFUSION RATE** ("Δ") are determined by the current rate:

Current Rate (units/hr)	Δ = Rate Change (units/hr)	2Δ = 2X Rate Change (units/hr)
≤ 1	0.25	0.5
>1-3	0.5	1
> 3	1	2

† **DISCONTINUE** insulin infusion . Check BG q 30 min; when BG is ≥ 90 mg/dL restart insulin infusion at 75% of most recent rate

SUPPLEMENTARY DATA

REFERENCES:

1. Osbak KK, Colclough K, Saint‐Martin C, Beer NL, Bellanné‐Chantelot C, Ellard S, Gloyn AL: Update on mutations in glucokinase (GCK), which cause maturity‐onset diabetes of the young, permanent neonatal diabetes, and hyperinsulinemic hypoglycemia. Human Mutation 2009;30:1512-1526

2. Inoue M, Sakuraba Y, Motegi H, Kubota N, Toki H, Matsui J, Toyoda Y, Miwa I, Terauchi Y, Kadowaki T, Shigeyama Y, Kasuga M, Adachi T, Fujimoto N, Matsumoto R, Tsuchihashi K, Kagami T, Inoue A, Kaneda H, Ishijima J, Masuya H, Suzuki T, Wakana S, Gondo Y, Minowa O, Shiroishi T, Noda T: A series of maturity onset diabetes of the young, type 2 (MODY2) mouse models generated by a large-scale ENU mutagenesis program. Hum Mol Genet 2004;13:1147-1157

3. Shoemaker AH, Zienkiewicz J, Moore DJ: Clinical assessment of HNF1A and GCK variants and identification of a novel mutation causing MODY2. Diabetes Res Clin Pract 2012;96:e36-39

4. Hattersley AT, Beards F, Ballantyne E, Appleton M, Harvey R, Ellard S: Mutations in the glucokinase gene of the fetus result in reduced birth weight. Nat Genet 1998;19:268-270

5. Gloyn AL, van de Bunt M, Stratton IM, Lonie L, Tucker L, Ellard S, Holman RR: Prevalence of GCK mutations in individuals screened for fasting hyperglycaemia. Diabetologia 2009;52:172-174

6. Gloyn AL: Glucokinase (GCK) mutations in hyper- and hypoglycemia: Maturity-onset diabetes of the young, permanent neonatal diabetes, and hyperinsulinemia of infancy. Human Mutation 2003;22:353-362

7. Li X, Hwu Ting T, Sheng H, Li Liang C, Shao Y, Jiang M, Xu A, Lin Y, Liu L: *Genetic and clinical characteristics of Chinese children with Glucokinase-maturity-onset diabetes of the young (GCK-MODY)*. 2018

8. Caetano LA, Jorge AA, Malaquias AC, Trarbach EB, Queiroz MS, Nery M, Teles MG: Incidental mild hyperglycemia in children: two MODY 2 families identified in Brazilian subjects. Arq Bras Endocrinol Metabol 2012;56:519-524

9. Alkorta-Aranburu G, Carmody D, Cheng YW, Nelakuditi V, Ma L, Dickens JT, Das S, Greeley SAW, Del Gaudio D: Phenotypic heterogeneity in monogenic diabetes: the clinical and diagnostic utility of a gene panel-based next-generation sequencing approach. Mol Genet Metab 2014;113:315-320