# Glucose Metabolism In Vivo in Four Commonly Used Inbred Mouse Strains

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**OBJECTIVE**—To characterize differences in whole-body glucose metabolism between commonly used inbred mouse strains.

**RESEARCH DESIGN AND METHODS**—Hyperinsulinemic-euglycemic (~8.5 mmol/l) and -hypoglycemic (~3.0 mmol/l) clamps were done in catheterized, 5-h-fasted mice to assess insulin action and hypoglycemic counter-regulatory responsiveness. Hyperglycemic clamps (~15 mmol/l) were done to assess insulin secretion and compared with results in perifused islets.

**RESULTS**—Insulin action and hypoglycemic counter-regulatory and insulin secretory phenotypes varied considerably in four inbred mouse strains. In vivo insulin secretion was greatest in 129X1/Sv mice, but the counter-regulatory response to hypoglycemia was blunted. FVB/N mice in vivo showed no increase in glucose-stimulated insulin secretion, relative hepatic insulin resistance, and the highest counter-regulatory response to hypoglycemia. In DBA/2 mice, insulin action was lowest among the strains, and islets isolated had the greatest glucose-stimulated insulin secretion in vitro. In C57BL/6 mice, in vivo physiological responses to hyperinsulinemia at euglycemia and hypoglycemia were intermediate relative to other strains. Insulin secretion by C57BL/6 mice was similar to that in other strains in contrast to the blunted glucose-stimulated insulin secretion from isolated islets.

**CONCLUSIONS**—Strain-dependent differences exist in four inbred mouse strains frequently used for genetic manipulation and study of glucose metabolism. These results are important for selecting inbred mice to study glucose metabolism and for interpreting and designing experiments. ***Diabetes*** **57:1790–1799, 2008**

The development of new mouse models has allowed investigators to address questions related to glucose metabolism in ways that were not previously possible. Use of inbred mouse strains and proliferation of techniques to produce genetic modifications have been invaluable in defining the role of select genes under physiological conditions. To rigorously examine complex physiological processes in vivo has required the development of new experimental approaches for the mouse and the adaptation of techniques previously used in larger animals. Important technical advancements, including surgical catheterization (1) and miniaturization of clamp techniques (2) for the mouse, have furthered our ability to dissect the physiology underlying insulin action, insulin secretion, and counter-regulation to insulin-induced hypoglycemia under well-controlled physiological conditions.

Mouse models produced through genetic modification have been generated in a variety of mouse strains. It is widely recognized that the background mouse strain can influence phenotypes. Several examples have been described where identical genetic mutations in different inbred mouse strains result in different phenotypes (3–5). These findings indicate that the contribution of the inbred strain genetic background to the phenotype is an important factor to consider when designing and interpreting experiments.

The goal of the current studies was to define the glucoregulatory phenotype of four commonly used inbred mouse strains under well-controlled conditions. We investigated insulin action using the hyperinsulinemic-euglycemic clamp, counter-regulatory response during an insulin-induced hypoglycemic clamp, and insulin secretion during a hyperglycemic clamp. Insulin secretion assessed in vivo was compared with insulin secretion in perifused islets.

## RESEARCH DESIGN AND METHODS

**In vivo mouse procedures.** All procedures are standard in the Vanderbilt Mouse Metabolic Phenotyping Center (MMPC; http://www.mmpc.org) and were approved by the Vanderbilt Animal Care and Use Committee. Male C57BL/6, 129X1/Sv, DBA/2, and FVB/N mice (The Jackson Laboratories) were purchased at 9 weeks of age and studied at 12 weeks of age. Housing was temperature (23°C) and humidity controlled on a 12-h light:dark schedule with mice given free access to food (Harlan Teklad LM-485, no. 7912) and water.

Surgical procedures have been described previously (2). Briefly, mice were anesthetized with sodium pentobarbital, and the carotid artery and jugular vein were catheterized. Free catheter ends were tunneled under the skin to the back of the neck, externalized, and sealed with steel plugs. Lines were flushed daily with ~50 μl saline containing 200 units/ml heparin and 5 mg/ml ampicillin. These methods permit arterial sampling from an indwelling catheter and are less stressful than cut-tail sampling (2). Mice ($n$ = 98) were individually housed and recovered for 5 days after surgery. Only mice returning to within ~10% of presurgical body weight were studied. Body composition was determined 1 day before experimentation using an mq10 NMR analyzer (Bruker Optics).

The arterial catheter was used for blood sampling, and the venous catheter was used for infusing in all protocols (2,6). Catheters were attached to a swivel ~1 h before the first infusion. Mice were unrestrained and not handled thereafter to minimize stress. The experimental period ($t$ = 0–120 min) began at ~1300 h with the infusion of insulin (Humulin R; Eli Lilly) in euglycemic and hypoglycemic clamps and glucose in hyperglycemic clamps. The steady-state period was defined by stable glycemia between $t$ = 80–120 min. Saline-washed erythrocytes were infused (5–6 μl/min) during the experimental period to prevent a >5% fall in hematocrit.

Blood samples were collected from the arterial catheter in tubes containing

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TABLE 1
Basal 5-h fasted data

| | BL6 | 129 | FVB | DBA |
|---|---|---|---|---|
| Body weight (g) | 22.1 ± 0.3* | 21.2 ± 0.3† | 24.2 ± 0.3 | 22.5 ± 0.5 |
| Muscle mass (% body wt) | 77 ± 1*‡§ | 72 ± 1 | 73 ± 1 | 70 ± 1 |
| Fat mass (% body wt) | 9 ± 1*‡§ | 13 ± 1¶ | 14 ± 1‖ | 18 ± 1 |
| Whole-blood glucose (mmol/l) | 9.1 ± 0.2‡§ | 7.1 ± 0.2†¶ | 9.1 ± 0.3 | 7.8 ± 0.2 |
| Plasma glucose (mmol/l) | 9.7 ± 0.4‡§ | 8.6 ± 0.3 | 9.2 ± 0.1 | 8.8 ± 0.1 |
| Blood glucose:plasma glucose | 0.96 ± 0.04‡ | 0.84 ± 0.01†¶ | 0.95 ± 0.02 | 0.90 ± 0.02 |
| Insulin (pmol/l) | 138 ± 24‡§ | 90 ± 12¶ | 114 ± 18‖ | 198 ± 12 |
| C-peptide (nmol/l) | 0.59 ± 0.06* | 0.59 ± 0.06† | 0.36 ± 0.06 | 0.46 ± 0.06 |
| Glucagon (ng/l) | 61 ± 7*‡ | 48 ± 3¶ | 39 ± 3 | 60 ± 6 |
| Corticosterone (ng/l) | 0.17 ± 0.02 | 0.23 ± 0.04 | 0.25 ± 0.05 | 0.17 ± 0.02 |
| Epinephrine (pmol/l) | 300 ± 55*‡ | <109†¶ | 775 ± 175‖ | 257 ± 44 |
| Norepinephrine (nmol/l) | 0.92 ± 0.14*§ | 0.89 ± 0.08†¶ | 1.24 ± 0.11 | 1.27 ± 0.19 |
| Glucose endo$R_a$ (mmol · kg$^{-1}$ · min$^{-1}$) | 0.10 ± 0.01 | 0.10 ± 0.01 | 0.12 ± 0.01 | 0.12 ± 0.01 |
| Glucose $R_d$ (mmol · kg$^{-1}$ · min$^{-1}$) | 0.11 ± 0.01 | 0.11 ± 0.01 | 0.12 ± 0.01 | 0.12 ± 0.01 |
| Fasting plasma glucose–to–insulin ratio | 100 ± 11§ | 107 ± 13¶ | 105 ± 18‖ | 44 ± 3.6 |
| HOMA-IR | 11.4 ± 1.7‡ | 4.9 ± 0.5†¶ | 7.7 ± 1.1 | 11.5 ± 0.9 |
| QUICKI | 0.47 ± 0.02*‡ | 0.31 ± 0.01 | 0.30 ± 0.01 | 0.34 ± 0.07 |

Data are means ± SE. Data from 12-week-old, 5-h-fasted C57BL/6J (BL6), 129X1/SvJ (129), FVB/NJ (FVB), and DBA/2J (DBA) male mice. Body weight, blood glucose, insulin, fasting glucose–to–insulin ratio, HOMA-IR, and QUICKI were compiled from basal samples during hyperinsulinemic-euglycemic (euglycemic), insulin-induced hypoglycemic (hypoglycemic), and hyperglycemic clamp experiments in chronically catheterized, conscious mice ($n$ = 27 for BL6; 25 for 129; 22 for FVB; and 24 for DBA mice). QUICKI and HOMA-IR were calculated using equations described in RESEARCH DESIGN AND METHODS. Epinephrine and norepinepherine were measured before hypoglycemic and hyperglycemic clamps ($n$ = 19 for BL6; 18 for 129; 15 for FVB; and 14 for DBA mice). Muscle mass, fat mass, glucagon, and corticosterone were measured before hypoglycemic clamps ($n$ = 8 for BL6; 7 for 129; 7 for FVB; and 7 for DBA mice). Endo$R_a$, $R_d$, and plasma glucose were determined from euglycemic clamp data ($n$ = 8 for BL6; 9 for 129; 7 for FVB; and 10 for DBA mice). Comparisons are *BL6 vs. FVB, †129 vs. FVB, ‡BL6 vs. 129, §BL6 vs. DBA, ¶129 vs. DBA, and ‖FVB vs. DBA, where statistical significance is established at $P < 0.05$.

EDTA and centrifuged, and plasma was stored at −20°C until analyzed. After the final blood sample in euglycemic clamps, animals were injected with a bolus of pentobarbital, and liver and gastrocnemius were dissected and frozen in liquid nitrogen. Blood glucose refers to arterial blood glucose measured using the glucose oxidase method.

**Euglycemic clamp.** A primed continuous [3-$^3$H]glucose infusion (5-μCi bolus and 0.05 μCi/min) was given at $t$ = −120 min to measure glucose turnover. The clamp was started at $t$ = 0 min with a continuous insulin infusion (24 pmol · kg$^{-1}$ · min$^{-1}$), and the [3-$^3$H]glucose was increased to 0.1 μCi/min to minimize changes in specific activity. Glucose (5 μl) was measured every 10 min, and euglycemia (8.5 mmol/l) was maintained using a variable glucose infusion rate (GIR). Samples (10 μl) to determine glucose specific activity were taken at $t$ = −15 and −5 min and every 10 min from $t$ = 80–120 min. Samples (50 μl) were taken to measure plasma insulin at $t$ = 0, 100, and 120 min.

**Hypoglycemic clamp.** At $t$ = 0 min, a constant insulin infusion (120 pmol · kg$^{-1}$ · min$^{-1}$) was started to induce hypoglycemia, and a variable GIR was used to maintain blood glucose at ~3.0 mmol/l. Blood glucose (5 μl) was measured at $t$ = −15, −5, 5, 10, 15, and 20 min and then every 10 min until $t$ = 120 min. Samples (250 μl) were taken to measure plasma insulin, glucagon, corticosterone, and catecholamines at $t$ = −15, 30, 60, and 120 min.

**Hyperglycemic clamp.** At $t$ = 0 min, a variable GIR was used to increase and maintain blood glucose at ~15.0 mmol/l. Blood glucose (5 μl) was measured at $t$ = −15, −5, 5, 10, 15, and 20 min and then every 10 min until $t$ = 120 min. Samples (50 μl) to measure plasma insulin were taken at $t$ = −15, −5, 5, 10, 15, and 20 min and then every 20 min until $t$ = 120 min. Samples (50 μl) to measure plasma C-peptide were taken at $t$ = −5, 15, 110, and 120 min. Samples (100 μl) to measure plasma catecholamines were taken at $t$ = −5 and 120 min.

**Islet isolation and perifusion.** Islets were hand isolated ($n$ = 4–6 mice/strain) as previously described (7,8). After overnight culture, ~25 islets per mouse were perifused with low (5.6 mmol/l) glucose in perifusion media (38.1 mmol/l sodium bicarbonate, 4.0 mmol/l L-glutamine, 1.0 mmol/l sodium pyruvate, 0.5% phenol red, 5.0 mmol/l HEPES, and 0.1% BSA in 1.0 l Dulbecco's modified Eagle's medium without glucose [pH 7.4]) for a 30-min period. Islets were then perifused with 16.7 mmol/l glucose (6 min), 5.6 mmol/l glucose (24 min), 16.7 mmol/l glucose + 45 μmol/l isobutylmethylxanthine (IBMX) (6 min), 5.6 mmol/l glucose (24 min), and 16.7 mmol/l glucose + 125 μmol/l tolbutamide (6 min). Three-minute fractions were collected, and the five fractions before glucose stimulation were used to determine baseline insulin secretion.

**Measurement of pancreatic islet mass.** Pancreatic insulin content ($n$ = 4–6 mice/strain) was calculated as previously described (9). Three sections from different regions of the pancreas ($n$ = 3–4 mice/strain) were stained for insulin and glucagon to determine total islet, β-cell, and α-cell area by integrated morphometry (9).

**Plasma hormones, glucose, and plasma radioactivity.** Plasma insulin (10), glucagon (10), epinephrine (11), norepinephrine (11), and corticosterone (12) were determined by the Vanderbilt MMPC. Plasma glucose and [3-$^3$H]glucose radioactivity was determined as described previously (2).

**Immunoblotting.** Protein content was determined for GLUT4, hexokinase II (HKII), total Akt, and Akt phosphorylated on serine 473 ($p$-Akt) in gastrocnemius from euglycemic clamp mice using standard methods. Total Akt and $p$-Akt were also measured in liver, and Akt activation was determined by the $p$-Akt–to–total Akt ratio. Glyceraldehyde-3-phosphate dehydrogenase was used as a standard.

**Statistical analyses and calculations.** Values are represented as means ± SE. Statistical analyses were conducted using repeated-measures ANOVA or $t$ tests when appropriate. Statistical significance was established at $P < 0.05$.

Endo$R_a$ and $R_d$ were determined using Steele's non–steady-state equations (13). Negative endo$R_a$ values were seen in all groups except FVB/N mice and were treated as such. Specific activities were constant during euglycemic-clamp steady state, suggesting that a modeling error would be minor. It is possible that negative endo$R_a$ in a steady state may result from normal analytical error and/or small variability in glucose infusate. The homeostatic model assessment of insulin resistance (HOMA-IR) and quantitative insulin sensitivity check (QUICKI) were calculated using the equations [$(G_0 \times I_0)$/405)] and [1/log $(G_0)$ + log $(I_0)$], where $G_0$ and $I_0$ refer to 5-h fasting plasma glucose and insulin (14). Disposition index was determined by the equation disposition index = $S_{IClamp}$ × insulin secreted, where "$S_{IClamp}$" is insulin sensitivity during the euglycemic-clamp steady state and "insulin secreted" is measured during the 120-min hyperglycemic clamp. $S_{IClamp}$ was defined as $M/(G \times \Delta I)$ (14), where $M$ is the steady-state GIR, $G$ is steady-state glucose, and $\Delta I$ is the difference between basal and steady-state insulin.

## RESULTS

**Basal condition.** Basal data from 5-h-fasted mice are shown in Table 1. C57BL/6 mice had the highest blood glucose and glucagon, whereas 129X1/Sv mice had the lowest blood glucose and insulin. FVB/N mice had the

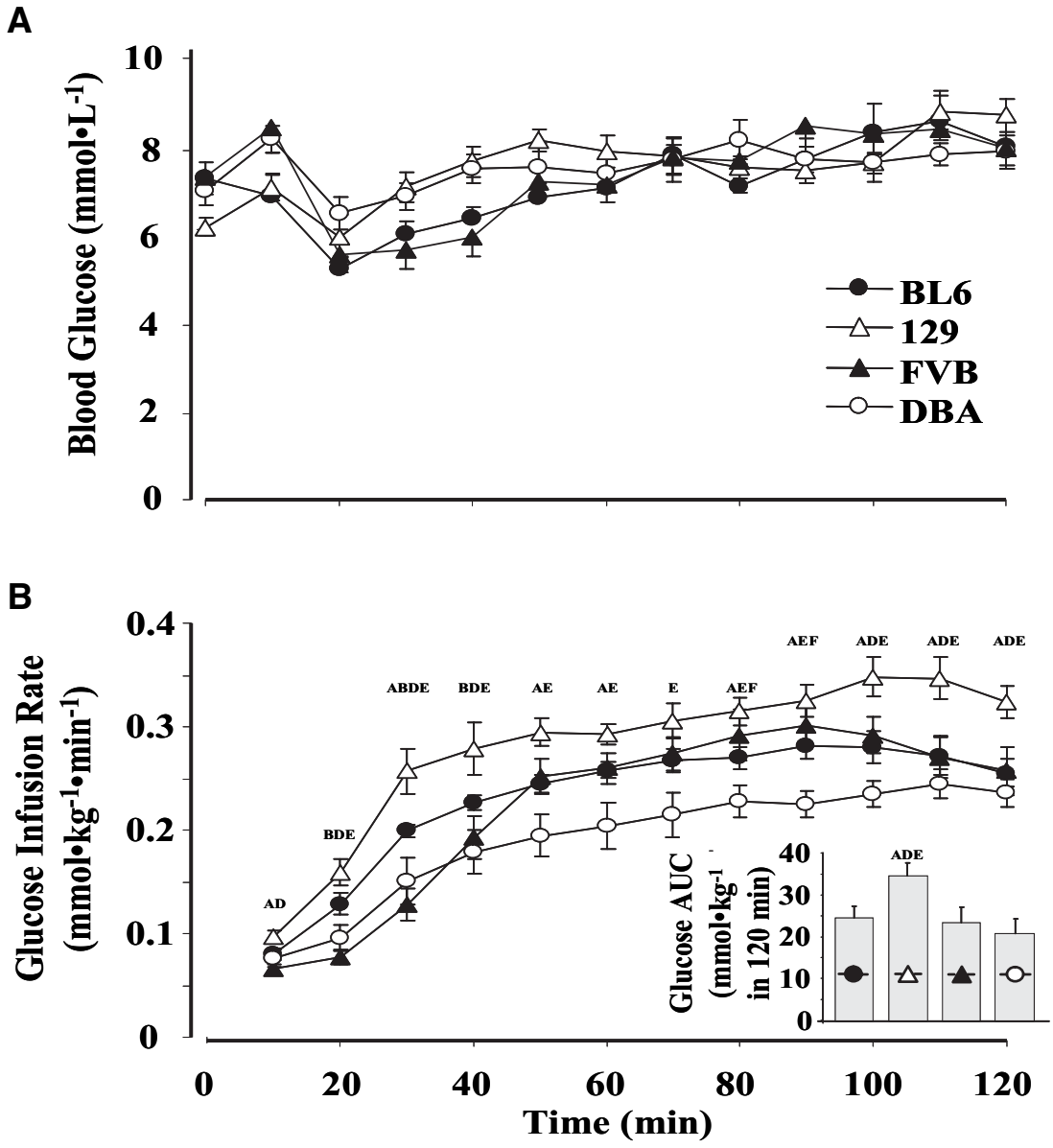


**FIG. 1. Whole-blood glucose (*A*; mmol/l) and GIR (*B*; mmol · kg$^{-1}$ · min$^{-1}$) during 120 min (*inset* shows glucose AUC [mmol/kg in 120 min]) in euglycemic-hyperinsulinemic clamp experiments in 5-h-fasted, surgically catheterized C57BL/6 (BL6; $n = 8$), 129X1/Sv (129; $n = 9$), FVB/N (FVB; $n = 7$), and DBA/2 (DBA; $n = 10$) male mice. A 96 pmol/kg insulin bolus was given at $t = 0$ min followed by a 24 pmol · kg$^{-1}$ · min$^{-1}$ constant infusion, and euglycemia (~8.5 mmol/l) was maintained during the clamp using a variable GIR. Basal samples were taken at $t = -15$ and 0 min, and the mean is represented as $t = 0$ min. All values are means ± SE, and comparisons are [A]C57BL/6 versus 129X1/Sv, [B]C57BL/6 versus FVB/N, [C]C57BL/6 versus DBA/2, [D]129X1/Sv versus FVB/N, [E]129X1/Sv versus DBA/2, and [F]FVB/N versus DBA/2, where statistical significance is established at $P < 0.05$ and differences are noted above each time point for clarity.**

highest epinephrine and lowest glucagon, whereas DBA/2 mice had the highest fat mass and insulin. There were also strain differences in the ratio of whole-blood glucose to plasma glucose, suggesting differences in erythrocyte glucose transport kinetics. Basal hematocrit was similar between strains.

**Insulin action.** Insulin action was determined from the response to a constant insulin infusion. The GIR required to maintain euglycemia was highest in 129X1/Sv mice, lowest in DBA/2 mice, and similar in FVB/N and C57BL/6 mice (Fig. 1*B*; Table 2). However, steady-state insulin and $\Delta\text{Insulin}_{\text{Clamp-Basal}}$ was higher in 129X1/Sv ($P < 0.05$) and DBA/2 ($P > 0.05$; NS) mice compared with C57BL/6 and FVB/N mice (Table 2). When GIR was normalized to steady-state insulin, insulin action was lower in DBA/2 mice compared with 129X1/Sv, C57BL/6, and FVB/N mice (Table 2). This relative insulin resistance in DBA/2 mice was consistent with higher fasting insulin (Table 1).

Hyperinsulinemia fully suppressed endo$R_a$ in 129X1/Sv, C57BL/6, and DBA/2 mice, but endo$R_a$ was incompletely suppressed in FVB/N mice (Table 2). Steady-state $R_d$ was lowest in DBA/2 mice, intermediate in C57BL/6 mice, and highest in 129X1/Sv and FVB/N mice (Table 2). $R_d$ normalized to steady-state insulin levels was also lower in DBA/2 mice compared with 129X1/Sv, C57BL/6, and DBA/2 mice, which is consistent with normalized GIR results (Table 2).

There were no strain-dependent differences in gastrocnemius GLUT4 or HKII protein (Fig. 2*A* and *B*). Total Akt was also similar in muscle and liver from each strain. There were no differences in gastrocnemius Akt activation (Fig. 2*C*). However, hepatic Akt activation was lower in FVB/N mice compared with 129X1/Sv, C57BL/6, and DBA/2

TABLE 2
Hyperinsulinemic-euglycemic clamp data

| | BL6 | 129 | FVB | DBA |
|---|---|---|---|---|
| GIR (mmol · kg$^{-1}$ · min$^{-1}$) | 0.30 ± 0.01* | 0.37 ± 0.02†‡ | 0.32 ± 0.02 | 0.26 ± 0.01 |
| Insulin (pmol · l$^{-1}$) | 338 ± 29§ | 420 ± 37 | 360 ± 34¶ | 472 ± 59 |
| $\Delta$Insulin$_{\text{Clamp-Basal}}$ (pmol · l$^{-1}$) | 230 ± 26 | 305 ± 45† | 211 ± 17 | 282 ± 47 |
| GIR/insulin (mmol · l$^{-1}$ · [kg · min$^{-1}$ · pmol$^{-1}$]$^{-1}$) × 10$^{-4}$ | 9.4 ± 0.8§ | 9.2 ± 0.8† | 9.4 ± 1.2¶ | 6.4 ± 0.9 |
| GIR/$\Delta$insulin$_{\text{Clamp-Basal}}$ (mmol · l$^{-1}$ · [kg · min$^{-1}$ · pmol$^{-1}$]$^{-1}$) × 10$^{-4}$ | 14.5 ± 1.9 | 14.9 ± 2.6 | 15.5 ± 1.4 | 15.4 ± 4.6 |
| Endo$R_a$ (mmol · kg$^{-1}$ · min$^{-1}$) | −0.03 ± 0.01‖ | −0.03 ± 0.02† | 0.02 ± 0.01¶ | −0.02 ± 0.01 |
| $R_d$ (mmol · kg$^{-1}$ · min$^{-1}$) | 0.27 ± 0.01*§‖ | 0.33 ± 0.02‡ | 0.34 ± 0.02¶ | 0.24 ± 0.01 |
| $R_d$/insulin (mmol · L$^{-1}$ · [kg · min$^{-1}$ · pmol$^{-1}$]$^{-1}$) × 10$^{-4}$ | 8.4 ± 0.7§ | 8.3 ± 0.7† | 10.2 ± 0.5¶ | 5.5 ± 0.8 |
| $\Delta$Insulin$_{\text{Clamp-Basal}}$/insulin infusion (kg · min · L$^{-1}$) | 9.6 ± 1.1 | 12.7 ± 1.9† | 8.8 ± 0.7 | 10.6 ± 2.3 |

Data are means ± SE. Steady-state data during 120-min hyperinsulinemic-euglycemic clamp experiments (24 pmol · kg$^{-1}$ · min$^{-1}$ insulin) in chronically catheterized, conscious, 5-h-fasted C57BL/6 (BL6; $n$ = 8), 129X1/Sv (129; $n$ = 9), FVB/N (FVB; $n$ = 7), and DBA/2 (DBA; $n$ = 10) male 12 week-old mice. Endo$R_a$ and $R_d$ are determined as described in RESEARCH DESIGN AND METHODS. $\Delta$Insulin $_{\text{Clamp-Basal}}$/insulin infusion is an assessment of insulin clearance. Steady-state values are the mean of five samples taken every 10 min from $t$ = 80–120 min. Comparisons are *BL6 vs. 129, †129 vs. FVB, ‡129 vs. DBA, §BL6 vs. DBA, ¶FVB vs. DBA, and ‖BL6 vs. FVB, where statistical significance is established at $P <$ 0.05.

mice (Fig. 2*D*). This is consistent with incomplete suppression of endo$R_a$ in FVB/N mice.

**Hypoglycemic counter-regulation.** Steady-state insulin was elevated to similar levels (1,974 ± 120, 2,262 ± 270, 2,220 ± 162, and 1,962 ± 126 pmol/l) in C57BL/6, 129X1/Sv, FVB/N, and DBA/2 mice, respectively, during hypoglycemic clamps. Steady-state GIR required to maintain hypoglycemia was lowest in FVB/N mice compared with similar rates in 129X1/Sv, C57BL/6, and DBA/2 mice (Fig. 3*A* and *B*). Total glucose requirement was highest in 129X1/Sv mice compared with C57BL/6, FVB/N, and DBA/2 mice (Fig. 3*B*, *inset*). The glucose requirement was inversely related to the counter-regulatory response.

In response to hypoglycemia, glucagon increased in C57BL/6, FVB/N, and DBA/2 mice to 4.0-, 9.9-, and 2.6-fold above basal and remained elevated (Fig. 3*C*). Glucagon was only transiently elevated in 129X1/Sv mice (2.3-fold above basal). Corticosterone were transiently elevated in DBA/2 mice but did not increase in C57BL/6, 129X1/Sv, and FVB/N mice (Fig. 3*D*). Epinephrine was increased in C57BL/6, FVB/N, and DBA/2 mice but was below the limits of detection in 129X1/Sv mice (Fig. 3*E*). Norepinephrine was increased in each strain in the hypoglycemic clamp (Fig. 3*F*). However, in FVB/N, the increase was no more so than during the hyperglycemic clamp at $t$ = 120 min.

**Insulin secretion.** Insulin secretion in response to hyperglycemia was assessed in vivo and compared with isolated islets to discern intra-islet mechanisms relative to whole-body physiology. Blood glucose was increased and maintained at ~15 mmol/l (Fig. 4*A*). The GIR was highest in 129X1/Sv mice, intermediate in C57BL/6 and DBA/2 mice, and lowest in FVB/N mice (Fig. 4*B*). Insulin was increased in C57BL/6, 129X1/Sv, and DBA/2 mice but not in FVB/N mice despite a similar hyperglycemic stimulus (Fig. 4*C*). The C-peptide responses paralleled insulin responses (Fig. 4*D*), and the insulin–to–C-peptide ratio did not differ between strains or change over time. Clamp norepinephrine remained similar to basal levels in C57BL/6, 129X1/Sv, and DBA/2 mice (0.78 ± 0.16, 1.08 ± 0.19, and 1.72 ± 0.60 nmol/l) but was significantly increased in FVB/N mice (2.86 ± 0.58 nmol/l). Epinephrine was unchanged from basal in C57BL/6 and DBA/2 mice (331 ± 38 and 318 ± 25 pmol/l), whereas FVB/N levels were increased to 2,038 ± 552 pmol/l. Epinephrine was below the limit of detection in 129X1/Sv mice even during hypoglycemia.

In isolated perifused islets, basal insulin secretion was 0.48 ± 0.18, 0.30 ± 0.18, 0.42 ± 0.06, and 1.20 ± 0.60 pmol/min in C57BL/6, 129X1/Sv, FVB/N, and DBA/2 mice, indicating strain-dependent differences (Fig. 5). Insulin secretory response to perifusion with high glucose, high glucose + isobutylmethylxanthine, and high glucose + tolbutamide was highest in DBA/2 mice compared with other strains (Fig. 5). Thus, the response to high glucose was C57BL/6 < 129X1/Sv < FVB/N < DBA/2. A similar response to isobutylmethylxanthine was seen in C57BL/6, 129X1/Sv, and FVB/N mice, whereas the response in DBA/2 mice was significantly higher. The response to tolbutamide in C57BL/6, 129X1/Sv, FVB/N, and DBA/2 mice was similar to the response to high glucose, suggesting little difference in $K_{ATP}$ channel closure or sensitivity to the drug between strains.

**Disposition index.** Disposition index is an empirical measure of glucose tolerance integrating insulin secretion and insulin sensitivity. Disposition index was calculated

TABLE 3
Pancreatic islet mass in four mouse strains

| | BL6 | 129 | FVB | DBA |
|---|---|---|---|---|
| Pancreatic insulin content (nmol/g pancreas) | 25.8 ± 3.1*† | 10.2 ± 1.4‡ | 17.2 ± 0.9‡ | 13.7 ± 1.2 |
| Insulin content in isolated islets (pmol/islet equivalent) | 10.6 ± 0.5*† | 7.9 ± 0.6‡ | 11.9 ± 0.3‡ | 7.9 ± 0.3 |
| Total islet mass (mg) | 2.5 ± 0.3 | 1.8 ± 0.1 | 2.8 ± 0.4 | 3.4 ± 0.2 |
| β-Cell mass (mg) | 2.3 ± 0.3 | 1.5 ± 0.1 | 2.6 ± 0.4 | 3.0 ± 0.2 |
| α-Cell mass (mg) | 0.20 ± 0.04 | 0.23 ± 0.03 | 0.18 ± 0.03 | 0.38 ± 0.05 |

Data are means ± SE. Total pancreatic insulin content, islet insulin content in isolated islets (normalized for size [islet equivalent]), total islet mass, β-cell mass, and α-cell mass from 12-week-old male C57BL/6 (BL6), 129X1/Sv (129), FVB/N (FVB), and DBA/2 (DBA) mice ($n$ = 4–6 mice/group). Comparisons are *BL6 vs. 129, †BL6 vs. FVB, ‡129 vs. DBA, and §FVB vs. DBA, where statistical significance is established at $P <$ 0.05.

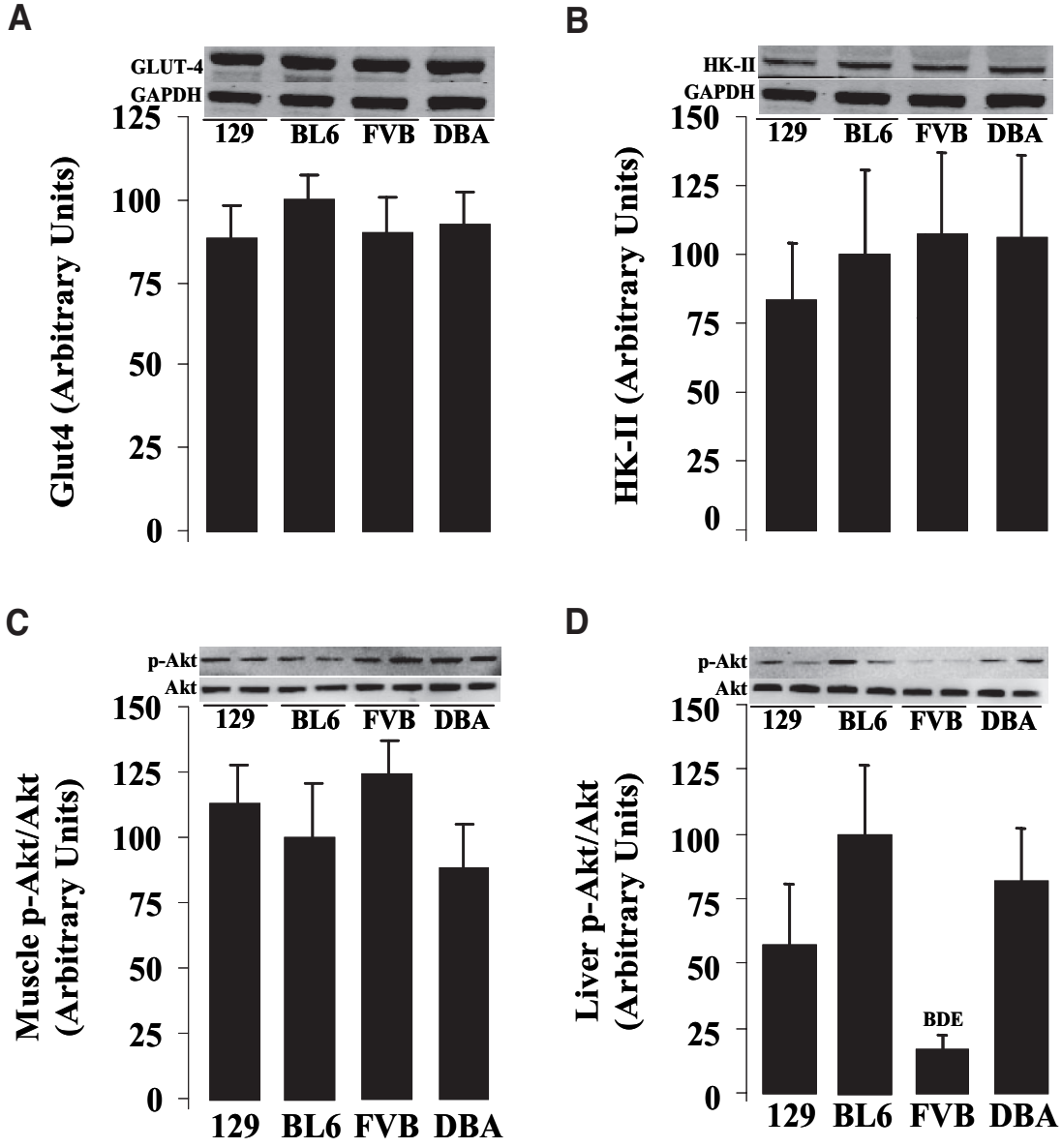


**FIG. 2. Comparison of Glut4 (*A*), HKII (*B*), and *p*-Akt/total Akt (Akt) protein content in gastrocnemius muscle (*C*) and liver (*D*) tissue from C57BL/6 (BL6; $n = 8$), 129X1/Sv (129; $n = 9$), FVB/N (FVB; $n = 7$), and DBA/2 (DBA; $n = 10$) mice after 120-min euglycemic-hyperinsulinemic (24 pmol · kg$^{-1}$ · min$^{-1}$) clamp experiments. GLUT4 and HKII were normalized to glyceraldehyde-3-phosphate dehydrogenase protein content. Mice were anesthetized at $t = 120$ min after the clamp using a bolus of pentobarbital, and tissues were quickly excised. Protein content was determined using methods described in RESEARCH DESIGN AND METHODS. All values are arbitrary units normalized to C57BL/6 mice and expressed as means ± SE. Comparisons are $^{B}$C57BL/6 versus FVB/N, $^{D}$129X1/Sv versus FVB/N, and $^{E}$129X1/Sv versus DBA/2, where statistical significance is established at $P < 0.05$.**

by combining insulin sensitivity ($S_I$) data from euglycemic clamps and insulin secretion data (insulin area under the curve [AUC]) from hyperglycemic clamps. Disposition index values were 1.28 ± 0.03, 2.07 ± 0.16, 0.06 ± 0.01, and 0.24 ± 0.02 in C57BL/6, 129X1/Sv, FVB/N, and DBA/2 mice, respectively, indicating that FVB/N mice had the lowest capacity to dispose of glucose.

## DISCUSSION

The goal in this study was to systematically analyze glucose metabolism in four representative inbred mouse strains using three clamp techniques to study in vivo physiology under well-controlled conditions. In C57BL/6, 129X1/Sv, FVB/N, and DBA/2 mice, we show strain-dependent differences in insulin action, hypoglycemic counterregulation, and insulin secretion. These four strains were selected for comparison based on recommendations of the National MMPC Steering Committee because these strains are frequently used in metabolic studies. In these experiments, glucoregulatory phenotype was assessed using in vivo euglycemic, hypoglycemic, and hyperglycemic clamps developed to study whole-body physiology in mice. This research emphasizes that genetic background is a critical factor to consider when designing and interpreting experiments. These results are important because in vivo clamp techniques are increasingly used to study physiology in mice, and this is the first published study to comprehensively address the contribution of genetic background to results obtained during in vivo clamp experiments.

While a number of previous studies have investigated phenotypic glucoregulatory differences between different

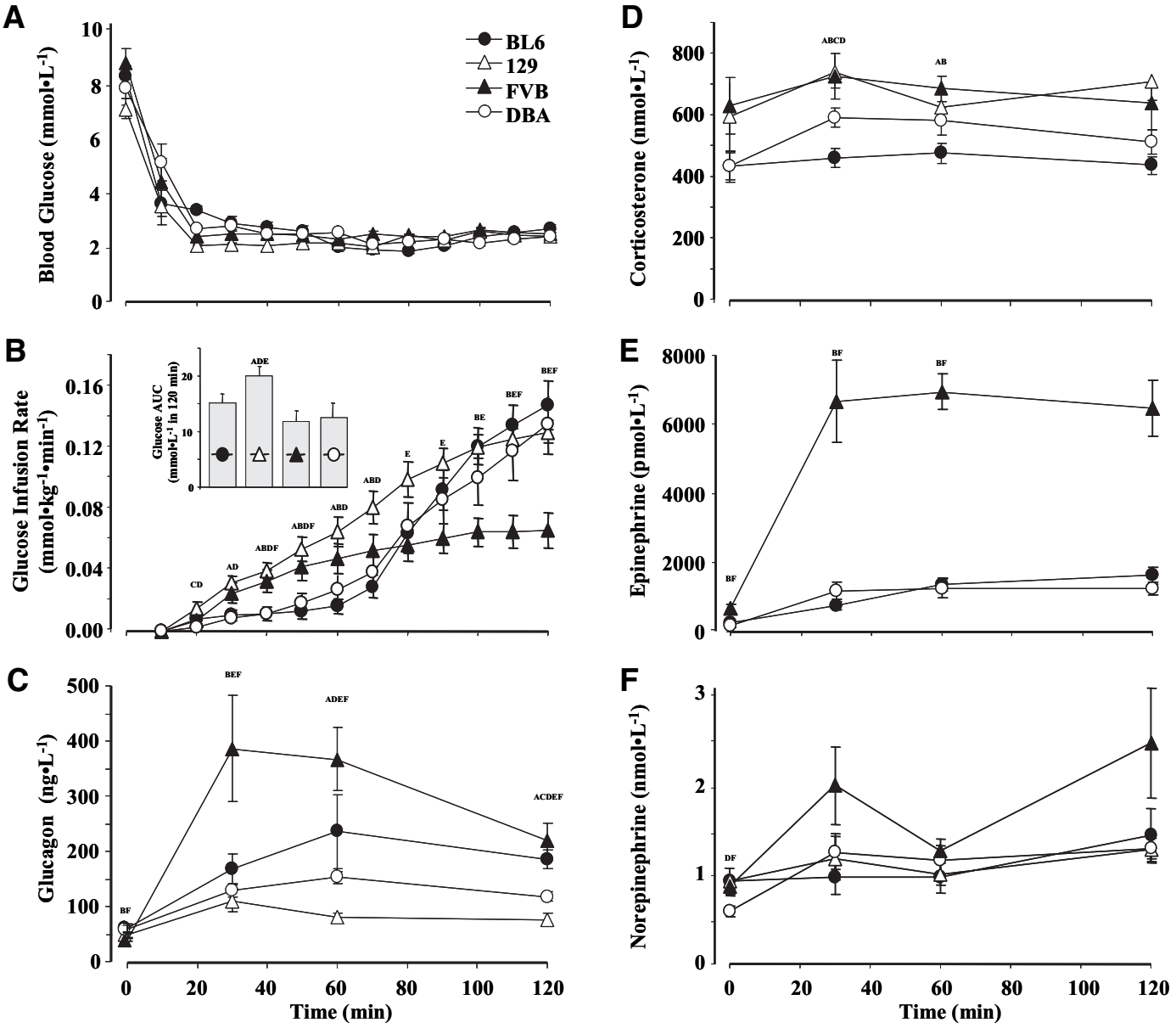


**FIG. 3. Data from 120-min insulin-induced hypoglycemic clamp experiments in 5-h-fasted, surgically catheterized C57BL/6 (BL6; $n = 8$), 129X1/Sv (129; $n = 9$), FVB/N (FVB; $n = 9$), and DBA/2 (DBA; $n = 10$) male mice. Insulin was infused at $t = 0$ min at 120 pmol · kg$^{-1}$ · min$^{-1}$, and whole-blood glucose (*A*; mmol · l$^{-1}$) was maintained at ~3.0 using a variable GIR (*B*; mmol · kg$^{-1}$ · min$^{-1}$). *Inset* in *B* depicts the glucose AUC during the 120-min experimental period determined by the trapezoidal method. The hypoglycemic counter-regulatory response was assessed by determining glucagon (*C*; ng/l), corticosterone (*D*; ng/l), epinephrine (*F*; pmol/l), and norepinephrine (*G*; nmol/l) at $t = 0$ (basal), 30, 60, and 120 min. Epinephrine levels in 129X1/Sv mice were below detection at all time points and were excluded from *F*. All values are means ± SE, and comparisons are [A]C57BL/6 versus 129X1/Sv, [B]C57BL/6 versus FVB/N, [C]C57BL/6 versus DBA/2, [D]129X1/Sv versus FVB/N, [E]129X1/Sv versus DBA/2, and [F]FVB/N versus DBA/2, where statistical significance is established at $P < 0.05$ and differences are noted above each time point for clarity.**

inbred strains (3–5,15–23), a key distinction in these studies is that techniques to study physiology in conscious, unstressed mice under well-controlled glycemic conditions were used. One previously published study used in vivo clamp techniques to examine differences between two different inbred mouse strains (4). This study assessed insulin action using methods (i.e., cut-tail blood sampling, large insulin prime, and overnight fast) previously shown to induce acute hepatic insulin resistance and higher catecholamines (2).

The majority of previous studies performed in vivo to study whole-body physiology have used insulin and glucose tolerance tests to study glucose metabolism (5,15,18–20). These assessments can be difficult to interpret because results are generally expressed as percent change relative to basal blood glucose or insulin levels, which may vary by strain or with genetic manipulation. Clamp methods are considered the gold standard for assessing glucose metabolism because glycemia is controlled, thus alleviating interpretation problems related to changes in blood glucose.

Euglycemic clamp results indicate strain-dependent differences in insulin action and highlight complexities of this method. GIR levels were different between strains, suggesting differences in insulin action, but steady-state insulin varied between strains. Normalizing GIR to clamp insulin accounts for differences and permits a more complete interpretation of insulin action. GIR normalized to

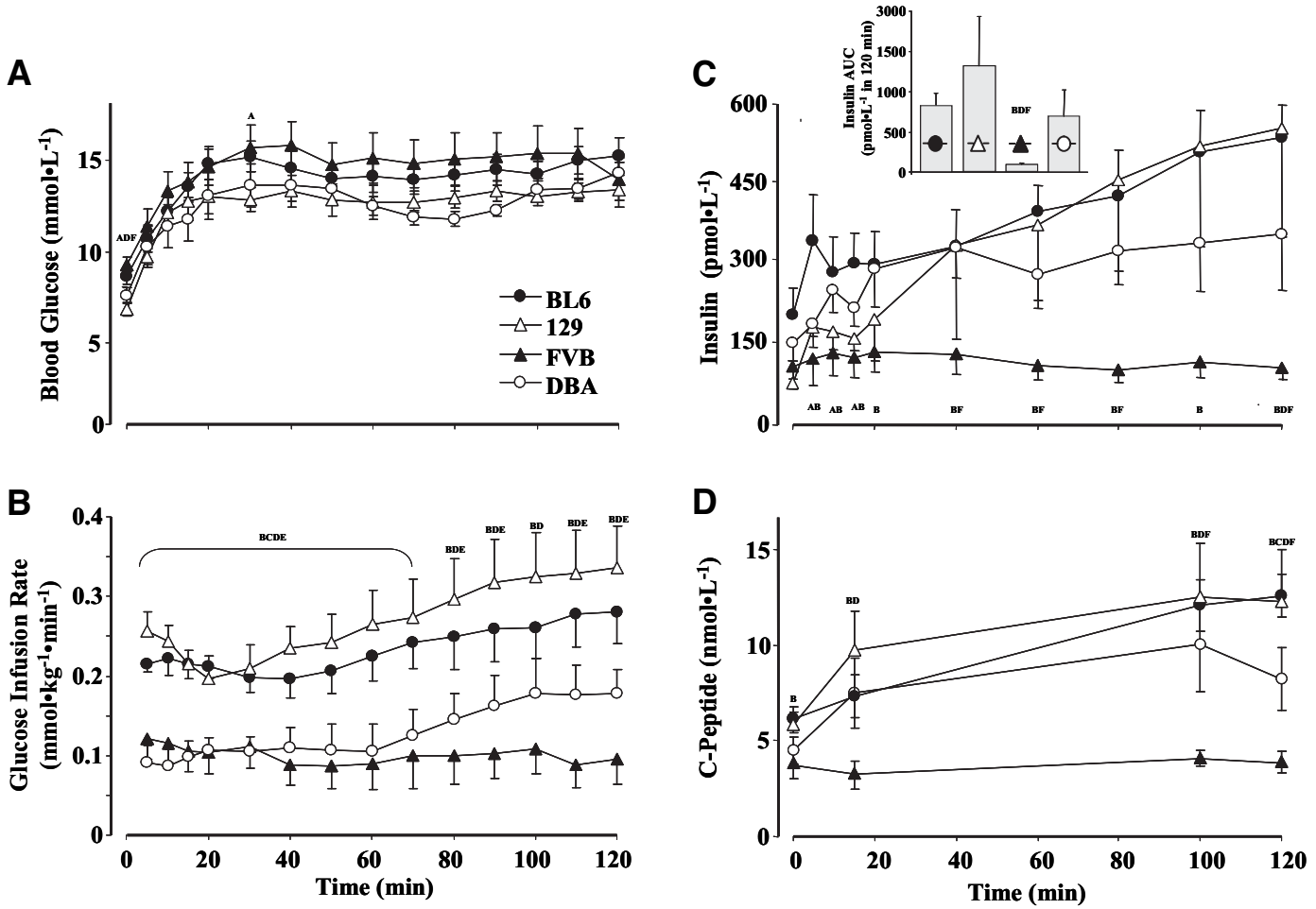


**FIG. 4. Data from 120-min hyperglycemic clamp experiments in 5-h-fasted, surgically catheterized C57BL/6 (BL6; $n = 8$), 129X1/Sv (129; $n = 9$), FVB/N (FVB; $n = 9$), and DBA/2 (DBA; $n = 10$) male mice. Whole-blood glucose (*A*; mmol/l) levels were elevated and maintained at 15 mmol/l using a variable GIR (*B*; mmol · kg$^{-1}$ · min$^{-1}$). *C*: Insulin (pmol/l) secretion was assessed at $t = 5$, 10, 15, 20, and then every 20 min from $t = 40$–120 min (*inset* shows insulin secretion AUC [pmol/l in 120 min]). *D*: C-peptide (nmol/l) was assessed at $t = 100$ and 120 min. Basal values ($t = 0$ min) represent the mean of two samples taken at $t = -15$ and $-5$ min. All values are means ± SE, and comparisons are [A]C57BL/6 versus 129X1/Sv, [B]C57BL/6 versus FVB/N, [C]C57BL/6 versus DBA/2, [D]129X1/Sv versus FVB/N, [E]129X1/Sv versus DBA/2, and [F]FVB/N versus DBA/2, where statistical significance is established at $P < 0.05$ and differences are noted above and below each time point for clarity.**

insulin indicates that DBA/2 mice are insulin resistant compared with 129X1/Sv, C57BL/6, and FVB/N mice. This is consistent with higher basal insulin in DBA/2 mice, which may reflect some degree of β-cell compensation. There were no strain-dependent differences in GIR normalized to $\Delta\text{Insulin}_{\text{Clamp-Basal}}$ (Table 2). The larger $\Delta\text{Insulin}_{\text{Clamp-Basal}}$ in 129X1/Sv mice compared with FVB/N mice suggests strain-dependent differences in insulin clearance. One might also speculate that differences in clamp insulin are due to insulin-mediated suppression of β-cell insulin secretion. It is common not to report insulin levels (24–26). The present study demonstrates that insulin must be reported to fully interpret results from clamp studies.

Insulin clamp GIR and $R_d$ were significantly correlated with QUICKI and HOMA-IR ($P < 0.01$). The correlation coefficients comparing GIR to QUICKI and HOMA-IR were 0.53 and −0.54, respectively. The correlation coefficients comparing $R_d$ to QUICKI and HOMA-IR were 0.53 and −0.50, respectively. These correlations were equally significant using GIR and $R_d$ normalized to insulin compared with QUICKI and HOMA-IR.

Euglycemic clamp studies also suggest tissue-specific differences in insulin action. $R_d$ was lower in DBA/2 mice compared with other strains. The lower $R_d$ in DBA/2 mice did not correspond with differences in GLUT4 or HKII protein content or Akt activation in skeletal muscle. Lower $R_d$ in DBA/2 mice did correspond to a higher fat mass, which is consistent with an inverse relationship between fat mass and peripheral insulin action. This relationship also exists in humans. It is likely that the higher fat mass in DBA/2 mice contributes to insulin resistance, but it is impossible to establish a causal relationship from these studies. Hyperinsulinemia did not fully suppress endo$R_a$ in FVB/N mice, indicating relative hepatic resistance to insulin. This corresponded with a lower activation of hepatic Akt. The insulin infusion used here was not ideal for resolving liver phenotypes because the dose was beyond the most sensitive region of insulin to endo$R_a$ dose-response curve (2). This was evident by negative endo$R_a$ values in all but FVB/N mice. An insulin dose <15 pmol · kg$^{-1}$ · min$^{-1}$ would better isolate hepatic insulin action phenotypes.

The results also indicate that the response to hypoglycemia is strain dependent. The hypoglycemic clamp, to our knowledge, had only been done in C57BL/6 mice (12,27). Our results indicate that the endocrine response is largely absent in 129X1/Sv mice compared with C57BL/6, FVB/N, and DBA/2 mice. In contrast, the endocrine response in

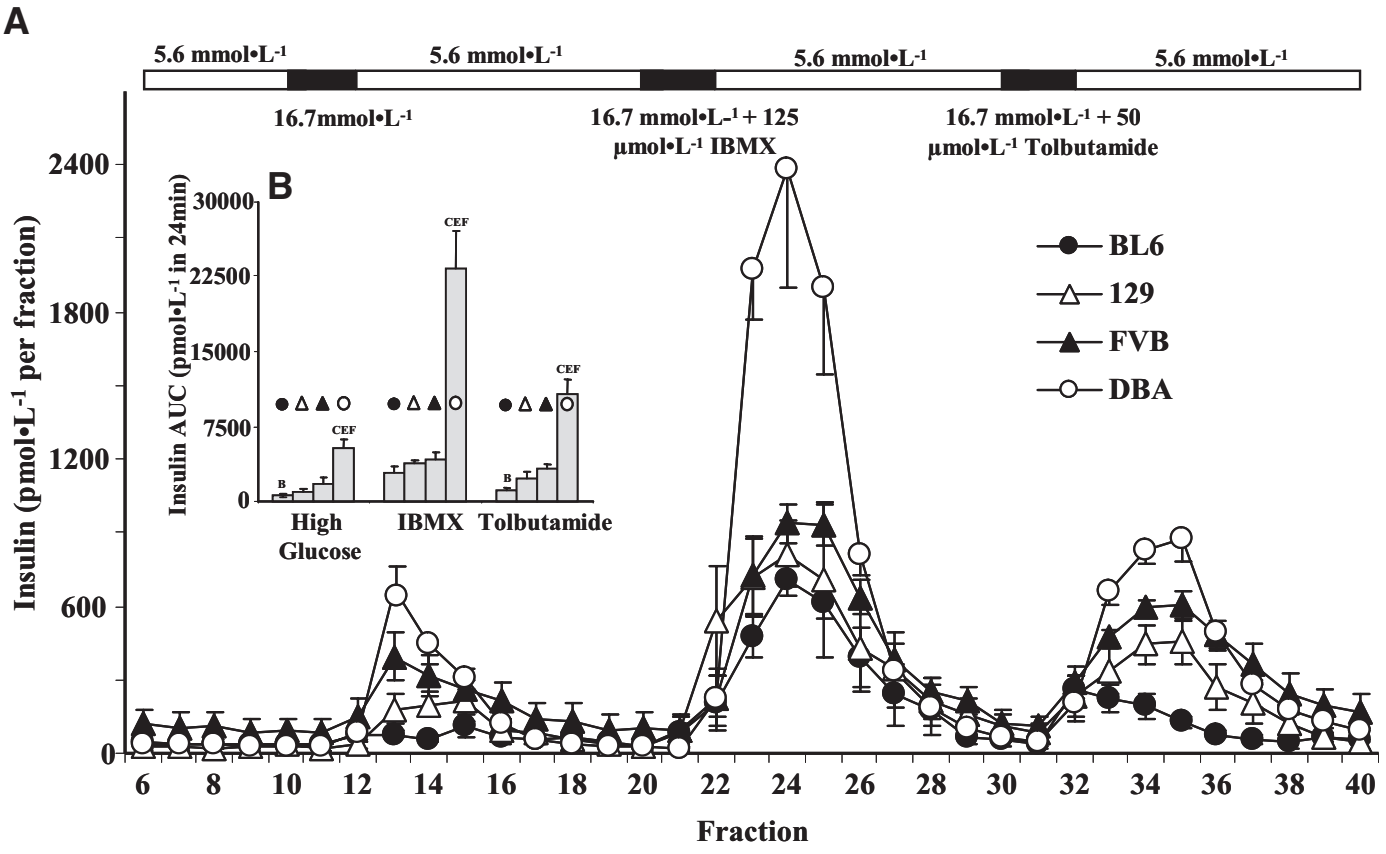


**FIG. 5. Insulin secretion (pmol/l) data from isolated islets of C57BL/6 (BL6; $n = 4$), 129X1/Sv (129; $n = 4$), FVB/N (FVB; $n = 4$), and DBA/2 (DBA; $n = 4$) mice perifused in fractions 6–10 with 5.6 mmol/l glucose; in fractions 11–12 with 16.7 mmol/l glucose followed by 5.6 mmol/l glucose in fractions 13–20; in fractions 21–22 with 16.7 mmol/l glucose + 125 µmol/l isobutylmethylxanthine followed by 5.6 mmol/l glucose in fractions 23–30; and in fractions 31–32 with 16.7 mmol/l + 50 µmol/l tolbutamide followed by 5.6 mmol/l glucose in fractions 33–40. *A*: Each fraction represents collection of effluent for 3 min and determination of insulin secreted in response to perifusion conditions in fractions 13–18, 23–28, and 33–38 and return to baseline in fractions 19–20, 29–30, and 39–40. *B*: Insulin secretion above basal (5.6 mmol · $l^{-1}$ glucose) AUC was determined using the trapezoidal method during the collection period for each treatment. All values are means ± SE, and comparisons are [A]C57BL/6 versus 129X1/Sv, [B]C57BL/6 versus FVB/N, [C]C57BL/6 versus DBA/2, [D]129X1/Sv versus FVB/N, [E]129X1/Sv versus DBA/2, and [F]FVB/N versus DBA/2, where statistical significance is established at $P < 0.05$. Symbols are not shown in *A* for clarity.**

FVB/N mice is far more potent compared with the other strains. This marked endocrine response in FVB/N mice did not correspond with lower GIR (compared with C57BL/6 and DBA/2) during the hypoglycemic clamp. This could be due to different sensitivities to glucagon and/or catecholamines, but it is more likely due to high insulin levels, which may mask the effects of counter-regulatory hormones. These findings are not only critical factors for hypoglycemic clamps but are also important issues in insulin tolerance tests used to estimate insulin action. The key metric in both is the insulin-induced fall in glucose. Insulin tolerance tests could be complicated by differences in basal glycemia or differences in the counter-regulatory response when comparing mixed-background mice or different strains.

The insulin secretory response to hyperglycemia further highlights phenotypic differences between strains and the complexity underlying the physiology of insulin secretion. Several groups have previously investigated differences in insulin secretion in vitro and in vivo using glucose tolerance tests in inbred mouse strains (15,19,20,22,23). Our measurements made in vivo using hyperglycemic clamps extend these results to fixed glycemic conditions. The objective was to match blood glucose between strains and quickly achieve hyperglycemia. The GIR is therefore dictated by the physiological response (i.e., blood glucose). The initial GIR is lower in FVB/N and DBA/2 mice compared with 129X1/Sv and C57BL/6 mice based on these glycemic responses (Fig. 4*B*). When an initial GIR similar to that in 129X1/Sv and C57BL/6 mice was used in FVB/N and DBA/2 mice, severe hyperglycemia occurred, often exceeding the upper limit of detection (27 mmol/l).

The failure of FVB/N mice to respond to glucose in vivo under clamp conditions is interesting in light of the intact in vitro response. Catecholamines were elevated in FVB/N mice compared with other strains and might explain the failure to increase insulin secretion in vivo (28). The robust insulin response in vivo in C57BL/6 mice contrasts with blunted secretion in vitro and previous work showing that C57BL/6 mice secrete less insulin in vivo compared with other strains (15,19,22,23). In previous studies, this has been associated with deletion of the nicotinamide nucleotide transhydrogenase gene in C57BL/6 mice (22,29). It is possible that there is long-term compensation for impaired insulin secretion in C57BL/6 mice (30). However, there were no profound differences in C57BL/6 pancreatic insulin content, islet insulin content, or β-cell mass.

The correlation coefficient between in vitro and in vivo insulin secretion in response to hyperglycemia was low ($r = -0.20$). Insulin content in the total pancreas and individual islets normalized for size (islet equivalent) was assessed to understand the mechanism for differences in insulin secretion in vivo and in isolated islets. There was

no systematic relationship between insulin content in isolated islets and insulin response (insulin secretion AUC) to high glucose in isolated islets ($r = 0.25$) or the insulin response to hyperglycemia in vivo ($r = 0.01$). There was also no relationship between pancreatic insulin content and insulin response to high glucose in isolated islets ($r = -0.39$) or in vivo ($r = -0.17$). This suggests that differences in secretion in isolated islet versus in vivo studies are due to differences in glucose sensing or stimulus/response coupling. These results highlight that unknown factors both intrinsic and extrinsic to islets contribute to these strain-dependent differences in insulin secretion and may complicate the interpretation of insulin secretion in vivo and in isolated islets.

An important observation is the strain-dependent differences in the ratio of whole-blood glucose to plasma glucose. This is an important consideration because clamp blood glucose may differ depending on whether whole-blood or plasma glucose is used. Also, using whole-blood glucose and plasma [3-$^3$H]glucose concentrations to calculate glucose specific activity will underestimate endo$R_a$. It is therefore important to use plasma glucose when calculating glucose turnover regardless of which method was used to clamp the mouse.

In summary, these results expose differences in glucose homeostasis in four commonly used mouse strains. Previous studies have documented the need to consider the contribution of the inbred strain to results from genetic manipulation on phenotype. Our results provide an important empirical reference for this under carefully controlled glycemic conditions. Our observations indicate that these four inbred mouse strains have unique underlying phenotypes in the basal condition and in response to clamp conditions. Because C57BL/6 mice are a common background strain used to study glucose metabolism, it is important to note that this strain has an intermediate physiological response to each of the three clamp experiments in comparison with 129X1/Sv, FVB/N, and DBA/2 mice. This is reassuring and suggests that C57BL/6 mice are a suitable model for studies of glucose homeostasis. Overall, these results demonstrate that it is critical to recognize the underlying phenotype of the inbred strain when performing metabolic testing on genetically modified mice and when comparing results within and between laboratories. These data are informative for selection of background strain, experimental design, and data interpretation.

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