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 perfused 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 120X1/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

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he 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 in 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 perfused 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, 120X1/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. 7012) 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, exteriorized, 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...
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‡§ | <100†¶ | 775 ± 175§ | 257 ± 44 |
| Norepinephrine (nmol/l) | 0.92 ± 0.14§§ | 0.80 ± 0.08†¶ | 1.24 ± 0.11 | 1.27 ± 0.19 |
| Glucose endoR_I (mmol · kg⁻¹ · min⁻¹) | 0.10 ± 0.01 | 0.10 ± 0.01 | 0.12 ± 0.01 | 0.12 ± 0.01 |
| Glucose R_I (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 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).

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 MIMP. Plasma glucose and [3-¹³C]glucose radioactivity were determined as described previously (2).

Immunoblotting. Protein content was determined for GLUT4, hexokinase II and 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 perfused with 16.7 mmol/l glucose (6 min), 5.6 mmol/l glucose (24 min), 16.7 mmol/l glucose + 45 μmol/l isobutylmethyamine (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.
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 (Table 2). However, steady-state insulin and ∆Insulin 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 endoR accessory in 129X1/Sv, C57BL/6, and DBA/2 mice, but endoR accessory was incompletely suppressed in FVB/N mice (Table 2). Steady-state Rd was lowest in FVB/N mice, intermediate in C57BL/6 mice, and highest in 129X1/Sv and DBA/2 mice (Table 2). Rq 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. 2A and B). Total Akt was also similar in muscle and liver from each strain. There were no differences in gastrocnemius Akt activation (Fig. 2C). However, hepatic Akt activation was lower in FVB/N mice compared with 129X1/Sv, C57BL/6, and DBA/2
mice (Fig. 2D). This is consistent with incomplete suppression of endoRₐ 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 ± 120 pmol/l) in C57BL/6, 129X1/Sv, FVB/N, and DBA/2 mice, respectively, during hyperglycemic 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. 3A and B). Total glucose requirement was highest in 129X1/Sv mice compared with C57BL/6 mice, and was lowest in DBA/2 mice (Fig. 3B). 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. 3C). 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. 3D). Epinephrine was increased in C57BL/6, 129X1/Sv, and DBA/2 mice but was below the limits of detection in 129X1/Sv mice (Fig. 3E). Norepinephrine was increased in each strain in the hypoglycemic clamp (Fig. 3F). 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. 4A). The GIR was highest in 129X1/Sv mice, intermediate in C57BL/6 and DBA/2 mice, and lowest in FVB/N mice (Fig. 4B). Insulin was increased in C57BL/6, 129X1/Sv, and DBA/2 mice but not in FVB/N mice despite a similar hyperglycemic stimulus (Fig. 4C).

The C-peptide responses paralleled insulin responses (Fig. 4D), and the insulin–to–C-peptide ratio did not differ between strains or change over time. Clamp norepinephrine remained similar to basal levels in 129X1/Sv mice compared with FVB/N, and DBA/2 mice but was significantly increased in FVB/N mice (0.78 ± 0.16, 1.08 ± 0.19, and 1.72 ± 0.60 pmol/l) but was significantly increased in FVB/N mice (2.86 ± 0.58 pmol/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 perfused 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 perfusion 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 Kᵦₐₐₚ 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.
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.

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**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 

- BC57BL/6 versus FVB/N,
- D129X1/Sv versus FVB/N, and
- E129X1/Sv versus DBA/2,

where statistical significance is established at $P < 0.05$. 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.

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While a number of previous studies have investigated phenotypic glucoregulatory differences between different
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 clamp insulin varied by strain or with genetic manipulation.
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 ΔInsulinclamp-Basal (Table 2). The larger ΔInsulinclamp-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 Rq 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 Rq to QUICKI and HOMA-IR were 0.53 and −0.50, respectively. These correlations were equally significant using GIR and Rq normalized to insulin compared with QUICKI and HOMA-IR.

Euglycemic clamp studies also suggest tissue-specific differences in insulin action. Rq was lower in DBA/2 mice compared with other strains. The lower Rq in DBA/2 mice did not correspond with differences in GLUT4 or HKII protein content or Akt activation in skeletal muscle. Lower Rq 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 endoRn 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 endoRn dose-response curve (2). This was evident by negative endoRn 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
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 (22,29). 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. 4B). 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 \([3-3H]\)glucose concentrations to calculate glucose specific activity will underestimate endo\(R_g\). 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 C57/BL/6 mice are a suitable model for studies of glucose homeostasis. This is reassuring and suggests that C57BL/6 mice are a suitable model for studies of glucose homeostasis in 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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