

Evaluating the glucose tolerance test in mice

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Andrikopoulos S, Blair AR, Deluca N, Fam BC, Proietto J. Evaluating the glucose tolerance test in mice. *Am J Physiol Endocrinol Metab* 295: E1323–E1332, 2008. First published September 23, 2008; doi:10.1152/ajpendo.90617.2008.—The objective of this study was to determine the optimal conditions under which to assess glucose tolerance in chow- and high-fat-fed C57BL/6J mice. Mice were fed either chow or high-fat diet for 8 wk. Variables tested were fasting duration (0-, 3-, 6-, and 24-h and overnight fasting), route of administration (intraperitoneal vs. oral) load of glucose given (2, 1, or 0.5 g/kg and fixed 50-mg dose), and state of consciousness. Basal glucose concentrations were increased in high-fat- compared with chow-fed mice following 6 h of fasting (9.1 ± 0.3 vs. 7.9 ± 0.4 mmol/l $P = 0.01$). Glucose tolerance was most different and therefore significant ($P = 0.001$) in high-fat-fed mice after 6 h of fasting ($1,973 \pm 96$ vs. $1,248 \pm 83$ mmol $\cdot$ l $^{-1}\cdot$ 120 min $^{-1}$). The difference in glucose tolerance was greater following an OGTT (142%), in contrast to an IPGTT, with a 127% difference between high fat and chow. We also found that administering 2 g/kg of glucose resulted in a greater level of significance ($P = 0.0008$) in glucose intolerance in high-fat- compared with chow-fed mice. A fixed dose of 50 mg glucose regardless of body weight was enough to show glucose intolerance in high-fat- vs. chow-fed mice. Finally, high-fat-fed mice showed glucose intolerance compared with their chow-fed counterparts whether they were tested under conscious or anesthetized conditions. We conclude that 2 g/kg glucose administered orally following 6 h of fasting is best to assess glucose tolerance in mice under these conditions.

intraperitoneal glucose tolerance test; oral glucose tolerance test; fasting; high-fat feeding; C57BL/6J

TYPE 2 DIABETES IS CHARACTERIZED BY GLUCOSE INTOLERANCE, which is contributed to by peripheral (muscle, fat, and liver) insulin resistance as well as islet β -cell dysfunction (17). The glucose tolerance test is used in clinical practice and research to identify individuals with normal or impaired glucose tolerance and patients with type 2 diabetes. It is important to acknowledge that the glucose tolerance test is the only means of identifying impaired glucose tolerance. The glucose tolerance test is performed with the individual drinking a 75-g flavored glucose drink following an overnight fast (an evening meal is allowed but nothing else thereafter).

The molecular and biochemical mechanisms contributing to type 2 diabetes are not fully known. In an effort to understand these mechanisms, researchers have increasingly used genetically modified mouse models in which a gene is up- or downregulated in a tissue-specific manner. As such, the glucose tolerance test has become one of the most widely used physiological tests to characterize transgenic and knockout

mouse models of diabetes. In the mouse, the glucose tolerance test is routinely performed following an overnight fast, with the food being removed around 4 PM and the glucose load administered via an intraperitoneal injection. However, mice are nocturnal feeders, with $\sim 70\%$ of their daily caloric intake occurring during the dark cycle (8), and their metabolic rate is much higher than humans. Therefore, an overnight fast is a comparatively long time compared with humans and more akin to starvation. Furthermore, the administration of glucose via an intraperitoneal injection means that there is no incretin response that is known to significantly potentiate the glucose-mediated insulin response. Finally, there is no consistency on the amount of glucose administered, with studies using 1 (5, 25, 27) or 2 g/kg (7, 15, 18, 23, 32) or both doses to determine glucose tolerance in their genetically modified mice (13, 29).

In fact, these inconsistencies are evident from a survey we conducted in 2007 on 100 articles, using the keywords “glucose tolerance and mouse,” where we found the following information: route of administration: in 73 articles the glucose was administered intraperitoneally, whereas in 24 articles oral glucose tolerance tests (OGTT) were performed [in 3 articles both OGTT and intraperitoneal glucose tolerance tests (IPGTT) were used]; fasting duration: in 73 studies animals were fasted for 12 h or longer (overnight), in five studies an 8- to 10-h fast was used, in 18 studies mice were fasted for 4–6 h, and in the remaining four studies either the time was not stated or the mice were not fasted; dose: in 51 articles a dose of 2 g/kg was reported, in 26 articles a dose of 1 g/kg was used, and in the remaining 23 articles doses ranging from 0.5 to 3 g/kg were reported; anesthesia: in five articles studies were done on anesthetized mice, in eight articles experiments were conducted on conscious animals, and in 87 articles either the state of consciousness was not reported or the articles referred to previous publications.

This has led to some conflicting data. For example, in a recent article the deletion of endoplasmic reticulum chaperone P58^{IPK} resulted in β -cell failure and diabetes, yet glucose tolerance (following an overnight fast with the bolus administered ip) was unchanged (20). Similarly, mice with a complete deletion of muscle glycogen synthase were reported to have improved glucose tolerance despite reduced muscle and fat glucose uptake during a euglycemic hyperinsulinemic clamp (26). These inconsistencies have prompted us to reevaluate the glucose tolerance test to determine the most appropriate fasting time, route of administration, and dose of glucose to administer. In addition, the impact of anesthesia on oral glucose tolerance was also assessed. The pathological model that we chose to work with was the C57BL/6J high-fat-fed mouse, as

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this is a widely used model to precipitate glucose intolerance (1, 2, 4, 10, 12, 31).

MATERIALS AND METHODS

Animals. Male C57BL/6J mice were obtained at 8 wk of age from the Walter and Eliza Hall Institute of Medical Research (Kew, Victoria, Australia) and were allowed to acclimatize to their surroundings for 3 wk. It is important to note that the substrain of C57BL/6J used throughout this study has the recently described 5-exon deletion in nicotinamide nucleotide transhydrogenase (7). The origin of these mice is the Jackson Laboratories, and this is denoted by the letter J in C57BL/6J. Artificial lighting was maintained on a 12:12-h light-dark cycle. Room temperature was kept constant at 21°C. At 11 wk of age, mice were randomly divided into the two diet groups, the control group receiving a diet containing (wt/wt) 7% fat, 18% protein, and 75% carbohydrate. The high-fat group received a diet containing (wt/wt) 60% fat, 18% protein, and 22% carbohydrate. Both diets were otherwise identical and manufactured by Glen Forrest Specialty Feeds (Glen Forrest, Western Australia, Australia). The composition of the diets has been reported previously (4, 11). Ethics approval was obtained from the Austin Health Animal Ethics Committee, and all experiments were in accordance with the guidelines for the care and use of laboratory animals specified by this committee.

Body weight and food intake. Mice were housed in pairs, and body weight and food intake were determined weekly for 8 wk during the midportion of the light cycle. Prewedged food was placed in the food hoppers and measured on a per-cage basis. Food intake was determined as grams consumed per day.

IPGTT/OGTT. The IPGTT and OGTT were performed following 8 wk of diet feeding, as described previously (21). Briefly, after a designated fasting period, animals were anesthetized with an intraperitoneal injection (100 mg/kg) of pentobarbitone sodium (Therapon, Burwood, Victoria, Australia), and a silastic catheter filled with heparinized saline (20 U/ml) was inserted into the left carotid artery. Mice also underwent a tracheotomy to aid with breathing. A bolus of glucose was either injected into the intraperitoneal cavity or delivered into the stomach by a gavage needle (20-gauge, 38 mm long curved, with a 21/4 mm ball end; Able Scientific, Canning Vale, Western Australia, Australia), and 200 μ l of blood was sampled at 0, 15, 30, 60, and 120 min for plasma glucose and insulin analyses. Blood was immediately centrifuged, and the plasma was separated and stored at -20°C until assayed. The red blood cells were resuspended in an equal volume of heparinized saline and reinfused into the animal via the carotid artery prior to the collection of the next blood sample to prevent anemic shock.

Experiment 1: fasting duration. To determine an appropriate level of fasting, 10 mice/group were deprived of food beginning at 8 AM for 0, 3, 6, and 24 h, at which time the mice were anesthetized and an IPGTT was performed as described above. In a separate group of animals, the fast was performed overnight (18 h), with the food withdrawn at ~4 PM. This was done for comparison, because the overnight fast is most widely used in animal studies assessing glucose tolerance.

Experiment 2: route of administration. After a 6-h fast, nine mice/group were subjected to either an IPGTT or an OGTT (as described above).

Experiment 3: dosage of glucose. Dosage of glucose was determined by giving seven mice/group a bolus of 2, 1, or 0.5 g/kg of glucose orally. In a separate group of mice, the dose of glucose delivered was 50 mg regardless of body weight.

Experiment 4: impact of anesthesia on oral glucose tolerance. To assess the impact of anesthesia on the measured difference in glucose tolerance between our two study groups, 6-h fasted chow- and high-fat-fed mice were either anesthetized using an intraperitoneal injection of pentobarbitone sodium (100 mg/kg) or maintained awake.

All mice were gavaged with 50 mg of glucose and blood for measurement of glucose was taken by tail vein sampling.

Experiment 5: glucose tolerance of six inbred mouse strains. Ten-week-old male C57BL/6J, FVB/N, nonobese diabetic (NOD), 129T2/SV, DBA/2, and New Zealand obese (NZO) mice that were being maintained on a normal chow diet were obtained from the Walter and Eliza Hall Institute of Medical Research. On the day of experimentation, mice were fasted for 6 h (beginning at 8 AM), and an OGTT was performed using a 2 g/kg glucose bolus.

Measurement of plasma glucose and insulin concentrations. Blood glucose concentrations were measured on a Medisense Optium glucometer. The glucose oxidase method was used to determine plasma glucose levels using a GM7 Analox glucose analyzer (Helena Laboratories, Mount Waverley, Victoria, Australia). Plasma insulin concentrations were determined by a commercially available radioimmunoassay specific for rodent insulin (Linco Research Immunoassay, St. Charles, MO).

Statistical analysis. Data are expressed as means $\pm$ SE. The trapezoidal rule was used to determine the area under the curve (AUC). Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as (fasting glucose level $\times$ fasting insulin level)/22.4. The level of statistical significance was determined using Student's two-tailed *t*-test when the difference between the means of two populations was considered. For differences in plasma glucose or insulin levels during the glucose tolerance tests, an ANOVA was performed and significance determined using Tukey's post hoc test. A *P* value <0.05 was considered statistically significant.

RESULTS

Male C57BL/6J mice only were used throughout this study. Mice on a high-fat diet gained significantly more weight than chow-fed mice (Table 1). Although the amount of food consumed was significantly less, high-fat-fed mice had an increase in daily caloric intake due to the higher energy content of the diet (Table 1).

To determine the effect of fasting on glucose tolerance, C57BL/6J chow- and high-fat-fed mice were fasted for 0, 3, 6, 24, and 18 (overnight) h, and an IPGTT was performed using 2 g/kg glucose (Fig. 1). Although there was a trend for increased basal plasma glucose and insulin levels, this was only significantly different after 6 and 24 h of fasting, indicating impaired fasting glucose (Table 2). It is important to note that fasting reduced basal plasma insulin levels in both chow- and high-fat-fed mice by three- and fivefold, respectively, in the 24-h-fasted mice compared with their respective controls, which were fasted for 0 h (Table 2). During the IPGTT, there was a significant increase in plasma glucose levels in high-fat-fed vs. control mice only after 6 h of fasting (Fig. 1E). This was also illustrated with the AUC measurement (Fig. 1K). Interestingly, plasma insulin levels during the IPGTT were also significantly elevated following 6 h of fasting (Fig. 1F) as well as after no fasting (Fig. 1B), indicative of insulin resistance, and this was reflected in the AUC data (Fig. 1L). There was no

Table 1. Body weight gain and daily food and energy intake (following a 6-h fast) of C57BL/6J mice fed a chow or high-fat diet for 8 wk

	Chow Diet	High-Fat Diet
Body weight gain, g	1.39 $\pm$ 0.01	3.32 $\pm$ 0.01*
Food intake, g/day	3.41 $\pm$ 0.01	2.20 $\pm$ 0.01*
Energy intake, KJ/day	54.87 $\pm$ 0.12	61.54 $\pm$ 0.25*

Data are presented as means $\pm$ SE; *n* = 40. **P* < 0.005 vs. chow diet.

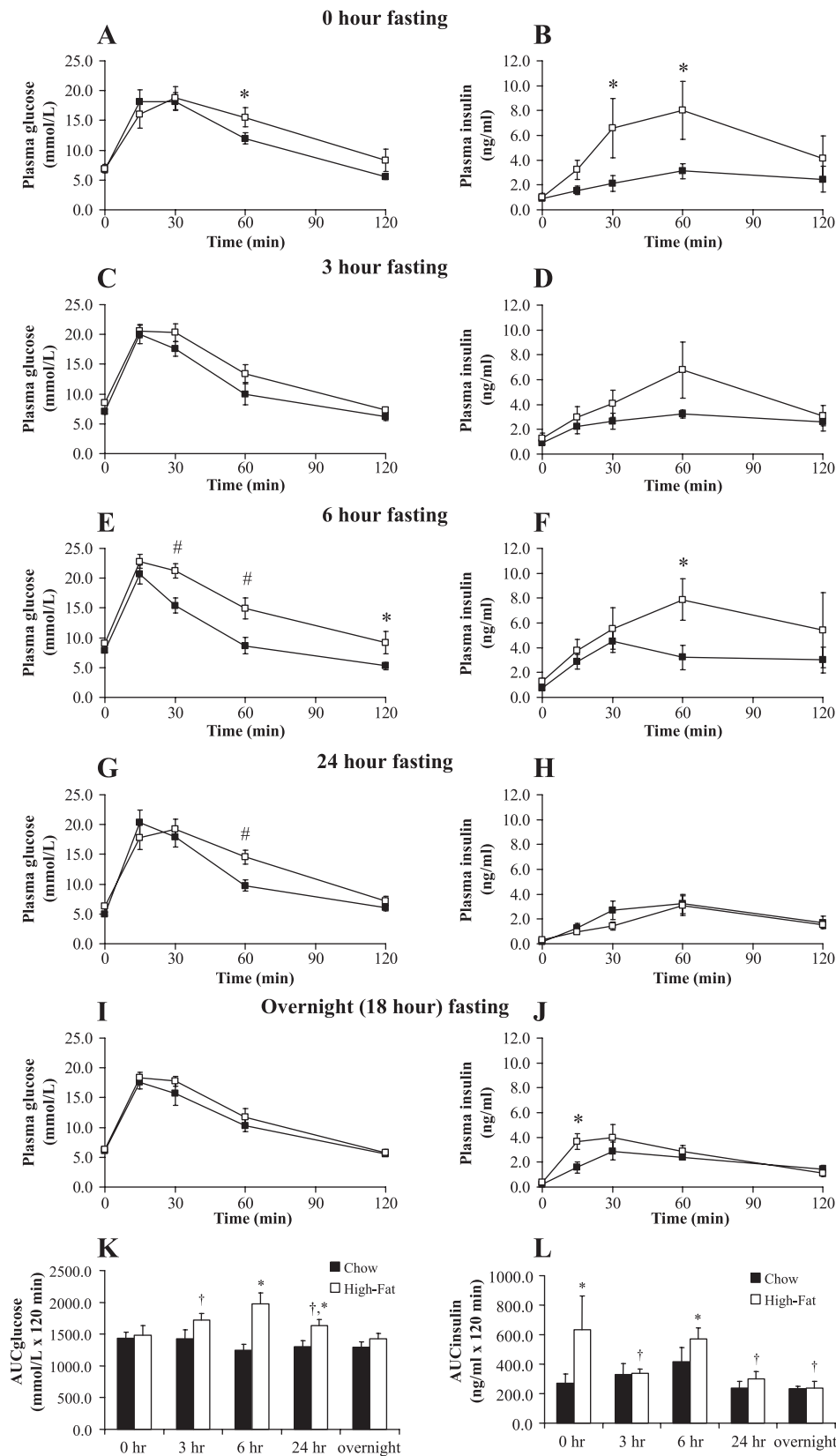


Fig. 1. Plasma glucose and insulin concentrations during the intraperitoneal glucose tolerance test (2 g/kg) following fasting for 0 (A and B), 3 (C and D), 6 (E and F), 24 (G and H), and 18 h (overnight; I and J) in C57BL/6J mice fed a standard chow (■) or high-fat (□) diet for 8 wk. Area under the curve for glucose (AUC_{glucose}; K) and insulin (AUC_{insulin}; L) was calculated using the trapezoidal rule. Results are means $\pm$ SE ($n = 8-10$). * $P < 0.05$; # $P < 0.005$, chow vs. high fat; † $P < 0.05$ vs. 6-h fasting high-fat diet group.

Table 2. Basal plasma glucose and insulin concentrations following the various fasting periods in C57BL/6J mice fed a chow or high-fat diet for 8 wk

	Chow Diet	High-Fat Diet
Plasma glucose levels, mmol/l		
0 h (8 AM)	6.73 ± 0.45	6.91 ± 0.70
3 h (8 AM)	7.03 ± 0.42	8.56 ± 0.39
6 h (8 AM)	7.88 ± 0.42	9.10 ± 0.30*
18 h (4 PM)	6.13 ± 0.22	6.28 ± 0.51
24 h (8 PM)	5.03 ± 0.32	6.26 ± 0.35*
Plasma insulin levels, ng/ml		
0 h (8 AM)	0.90 ± 0.69	1.03 ± 0.31
3 h (8 AM)	0.89 ± 0.17	1.27 ± 0.45
6 h (8 AM)	0.72 ± 0.13	1.28 ± 0.25*
18 h (4 PM)	0.22 ± 0.08	0.40 ± 0.08
24 h (8 AM)	0.17 ± 0.06	0.30 ± 0.11*

Data are presented as means ± SE. * $P < 0.05$. The start time of the fast is shown in parentheses.

effect of fasting on AUC_{glucose} (Fig. 1K) or AUC_{insulin} (Fig. 1L) in the chow-fed group. In the high-fat diet group the 6 h AUC_{glucose} was significantly higher than the 3- and 24-h AUC_{glucose} (Fig. 1K), whereas the 6-h AUC_{insulin} was significantly higher than the 3-h-, 24-h-, and overnight-fasted AUC_{insulin} (Fig. 1L).

When HOMA-IR was calculated, there was a significant increase in the high-fat group only after 6 h of fasting (Fig. 2A). There was also a significant positive correlation between AUC_{insulin} and HOMA-IR ($r = 0.77$, $P < 0.0001$) when all the data were considered. Furthermore, when only the 6-h fasting data were regressed, there was still a significant positive correlation between these two measurements of insulin sensitivity ($r = 0.73$, $P < 0.01$; Fig. 2B), whereas this association was lost after overnight (18 h) fasting ($r = 0.10$, $P = 0.76$). A significant positive correlation was also found between $AUC_{\text{insulin}}/AUC_{\text{glucose}}$ and HOMA-IR following a 6-h fast ($r = 0.69$, $P < 0.02$). Fasting overnight and for 24 h reduced HOMA-IR in both the chow- and high-fat-fed groups (Fig. 2A). On the basis of these data, we decided to conduct the subsequent experiments of this study by fasting the mice for 6 h.

The next step was to determine the effect of route of administration on glucose tolerance by delivering a 2 g/kg glucose bolus either by intraperitoneal injection or orally via gavage after a 6-h fast (Fig. 3). It is immediately clear that both the glucose and insulin excursions were significantly different depending on how glucose was delivered. In the IPGTT (Fig. 3A), there was a rise in plasma glucose levels >30 mmol/l, with the peak occurring at 15 min in chow-fed mice, whereas there was a sustained peak at 15 and 30 min in high-fat-fed mice. In contrast, during the OGTT (Fig. 3B), peak plasma glucose levels occurred at 15 min in both groups of animals, with a mean of 15.5 ± 0.6 mmol/l in the chow- and 24.4 ± 1.9 mmol/l in the high-fat-fed mice. Likewise, the plasma insulin levels during the IPGTT (Fig. 3C) showed a peak at 30 min with chow feeding, whereas there was a sustained peak at 30 and 60 min in the high-fat-fed group. On the contrary, during the OGTT (Fig. 3D) the peak plasma insulin levels occurred at 15 min in both groups of mice, with a significant increase at 15 and 30 min in the high-fat vs. chow group. When considering the AUC data, during both the IPGTT and OGTT the high-fat-fed group displayed glucose intolerance (Fig. 3E). Interest-

ingly, although there was a trend for increased AUC_{insulin} during the IPGTT, this parameter was significantly higher during the OGTT (Fig. 3F). The OGTT was associated with lower AUC_{glucose} (Fig. 3E) and AUC_{insulin} (Fig. 3F) for both chow- and high-fat-fed mice. For comparison, we performed OGTT following an overnight fast (Fig. 4). Although there was a significant increase in plasma glucose levels (Fig. 4A) and AUC_{glucose} (Fig. 4C) in the high-fat-fed mice compared with chow-fed mice, there was little difference in plasma insulin levels (Fig. 4B), and the AUC_{insulin} (Fig. 4D) was similar between the two groups of mice, confirming that a 6-h fast is more appropriate. These data suggest that delivering the glucose load orally following a 6-h fast results in significant increases in glucose and insulin profiles in high-fat-fed vs. chow-fed C57BL/6J mice.

To determine the effect of glucose dose on glucose tolerance in chow- and high-fat-fed mice, we performed OGTT following 6 h of fasting and compared 0.5 g/kg with 1–2 g/kg. Because the basal plasma glucose level was significantly higher in the high-fat-fed compared with chow-fed mice, all data in Fig. 5 are shown as percent change from basal. Plasma glucose levels were not different between chow- and high-fat-fed mice when challenged with either 0.5 (Fig. 5A) or 1 g/kg (Fig. 5C) glucose. It was only following the 2 g/kg dose that there was a separation in plasma glucose levels between the chow and high-fat diet group (Fig. 5E). Similarly, there were no differences in plasma insulin levels in response to either the 0.5 (Fig. 5B) or 1 g/kg (Fig. 5D) glucose dose between chow- and high-fat-fed mice. However, in response to 2 g/kg glucose there was a significant increase in insulin release in both groups of mice, with the high-fat animals showing a significantly higher response at 15 and 30 min compared with chow-fed mice (Fig. 5F). There was a significant increase in AUC_{glucose}

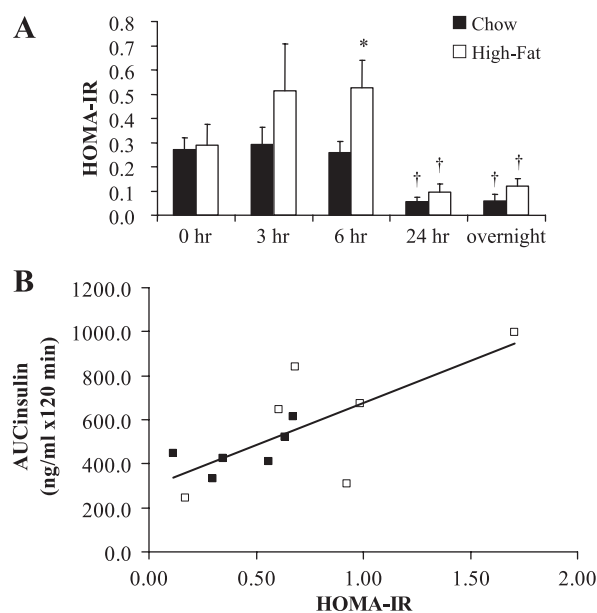


Fig. 2. A: homeostasis model assessment of insulin (HOMA-IR) following fasting for 0, 3, 6, 24, and 18 h (overnight) in C57BL/6J mice fed a standard chow (■) or high-fat (□) diet for 8 wk. B: correlation between HOMA-IR and AUC_{insulin} during the intraperitoneal glucose tolerance test (2 g/kg) in mice fasted for 6 h. Results are means ± SE ($n = 8-10$). * $P < 0.05$, chow vs. high fat; † $P < 0.05$ vs. the other time points in their respective diet groups.

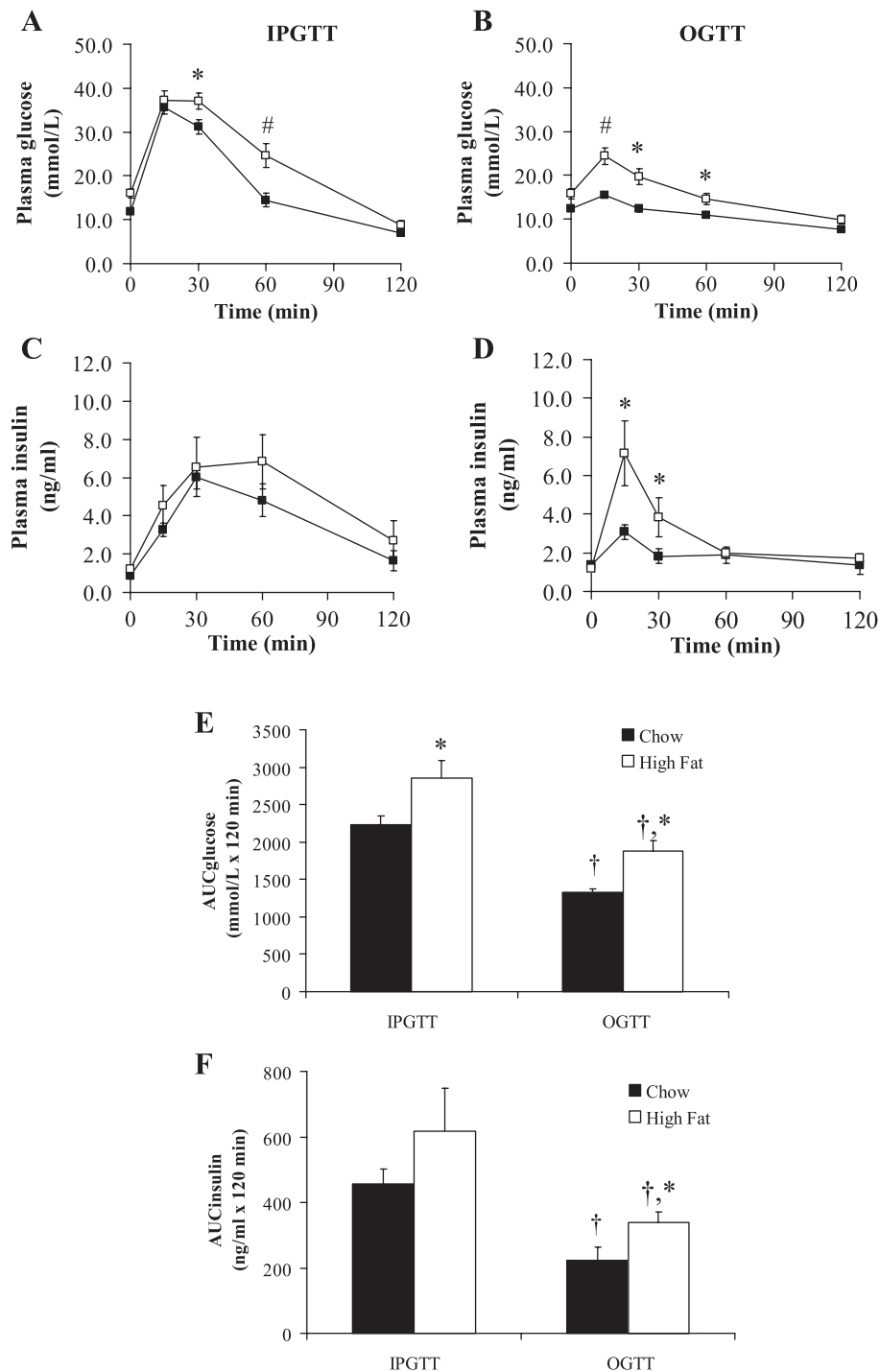


Fig. 3. Plasma glucose and insulin concentrations during the intraperitoneal glucose tolerance test (IPGTT; A and C) (2 g/kg) or oral glucose tolerance test (OGTT; B and D) (2 g/kg) following 6 h of fasting in C57BL/6J mice fed a standard chow (■) or high-fat (□) diet for 8 wk. AUC_{glucose} (E) and AUC_{insulin} (F) were calculated using the trapezoidal rule. Results are means $\pm$ SE ($n = 8-10$). * $P < 0.05$; # $P < 0.005$, chow vs. high fat; † $P < 0.05$ vs. IPGTT.

at the 2 g/kg glucose dose only (Fig. 5G). AUC_{insulin} was also increased only following the 2 g/kg glucose dose (Fig. 5H). AUC_{insulin} was significantly higher in the high-fat-fed 2 g/kg-treated mice compared with the other two doses in this diet group (Fig. 5H).

One can argue that it is not surprising that high-fat-fed mice have greater glucose excursions given that the glucose dose is given per kilogram body weight and they receive more glucose in absolute terms. Indeed, in our study, high-fat-fed mice were significantly heavier than chow-fed animals on the day of study (30.0 ± 0.8 vs. 27.9 ± 0.8 g, $n = 15$, $P < 0.05$). Therefore, we

conducted OGTT in which the chow- and high-fat-fed mice received a fixed dose of 50 mg of glucose (regardless of their body weight; data shown in Fig. 6). This dose was based on a 25-g mouse receiving 2 g/kg of glucose. There was a small but significant increase in both the glucose (Fig. 6A) and insulin excursion (Fig. 6B) during the OGTT in the high-fat- compared with chow-fed mice. This increase in the high-fat-fed mice was also seen in the AUC_{glucose} (Fig. 6C) and AUC_{insulin} (Fig. 6D) data.

In all of the above experiments, the mice were anesthetized before the glucose tolerance tests were conducted. To deter-

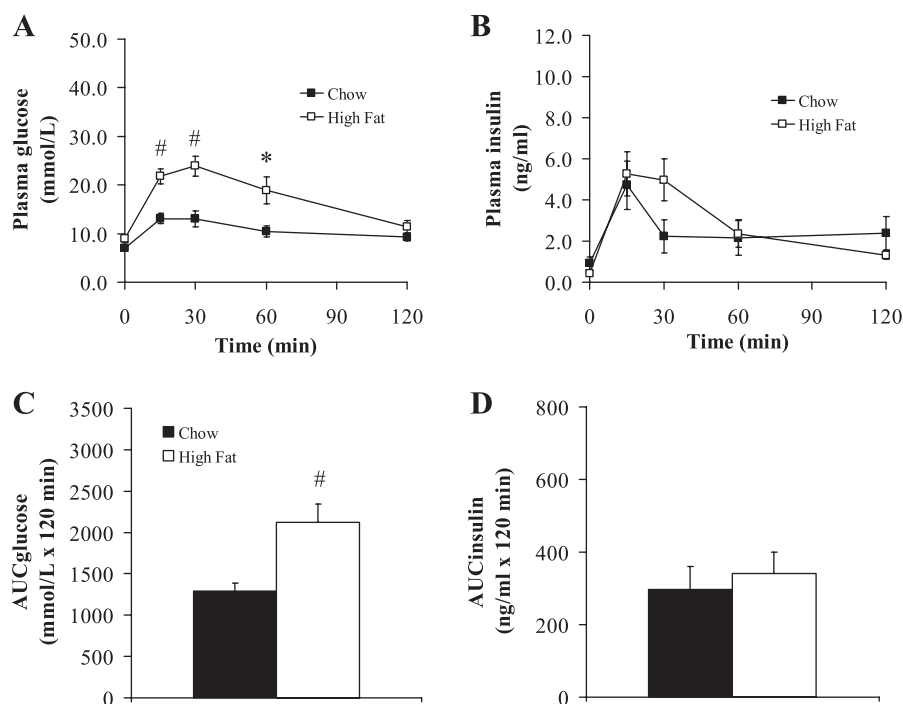


Fig. 4. Plasma glucose (A) and insulin (B) concentrations during the OGTT (2 g/kg) following overnight (18 h) fasting in C57BL/6J mice fed a standard chow (■) or high-fat (□) diet for 8 wk. AUC_{glucose} (C) and AUC_{insulin} (D) was calculated using the trapezoidal rule. Results are means $\pm$ SE (n = 8–10). * P < 0.05; # P < 0.005, chow vs. high fat.

mine whether consciousness would affect glucose tolerance, we conducted OGTT in mice that were either conscious or anesthetized, using a fixed dose of 50 mg glucose/mouse. For these experiments, we did not cannulate the mice and thus only measured blood glucose levels using a hand-held glucometer from blood sampled from the tail vein. It is clear from Fig. 7 that regardless of whether the mice were anesthetized (Fig. 7, A and C) or conscious (Fig. 7, B and C), high-fat feeding caused glucose intolerance compared with chow feeding.

From the above experiments, we concluded that 2 g/kg glucose delivered orally following a 6-h fast was the most appropriate set of conditions to determine glucose tolerance. To test this, we determined glucose tolerance in C57BL/6J, DBA/2, 129T2, FVB/N, the type 1 diabetes model NOD, and the obese and insulin-resistant model NZO. For clarity, we present the AUC data in Fig. 8. Interestingly, there was no difference in glucose tolerance (AUC_{glucose}) among any of these strains (Fig. 8A). However, we did find significant differences in AUC_{insulin}, with DBA/2 and NZO mice having higher insulin responses than the other strains, whereas the 129T2 mice had the lowest response (Fig. 8B).

DISCUSSION

In rodent research, the glucose tolerance test is the most widely used test to determine whether a genetically engineered (e.g., transgenic or knockout) or dietary-induced mouse is glucose intolerant and diabetic. As stated in the introductory section, it is evident that variables such as fasting duration, route, and amount of glucose administration, as well as state of consciousness, may have a large impact on the measured glucose tolerance in mice. Given the almost mandatory use of the glucose tolerance test, we are surprised that an attempt has not previously been made to validate the various variables of this test in mice.

As expected, our data showed that fasting duration had a major impact on both the plasma glucose and insulin levels. The overnight- and 24-h-fasted mice had significantly suppressed basal plasma glucose and insulin levels such that there were no differences between high-fat-fed and chow animals. Furthermore, there was a major suppressive effect on plasma insulin levels during the glucose tolerance test in both groups of animals, suggesting that overnight fasting in the mouse is a considerably long period of time. It has previously been shown that fasting C57BL/6J mice overnight (18 h) resulted in enhanced insulin sensitivity such that the rate of glucose disappearance was higher and endogenous glucose production was completely suppressed compared with 5 h of fasting, as assessed using the euglycemic hyperinsulinemic clamp (8). Liver glycogen levels have been shown to be nearly depleted, whereas liver triglycerides were sixfold higher following overnight fasting compared with 4–5 h fasting (8, 16). Furthermore, transgenic mice overexpressing GLUT4 (28) or hexokinase II (14) in muscle showed increased insulin sensitivity following an overnight fast but not after shorter fasting periods of 3–5 h. It is therefore not surprising to find in our study that the differences between chow- and high-fat-fed mice were less evident following a 24-h and the standard overnight (18 h) fast compared with shorter fasting times. In fact, we found that following 6 h of fasting there was a clear indication of glucose intolerance in the high-fat-fed compared with chow animals. Therefore, we chose this time point to conduct the subsequent tests of this study.

The fasting duration study allowed us to calculate HOMA-IR, a surrogate marker of insulin resistance. It is clear that prolonged fasting (overnight and 24 h) caused a significant reduction in HOMA-IR in both chow- and high-fat-fed mice such that there was no difference between the two groups. A significant difference was seen following 6 h of fasting, and

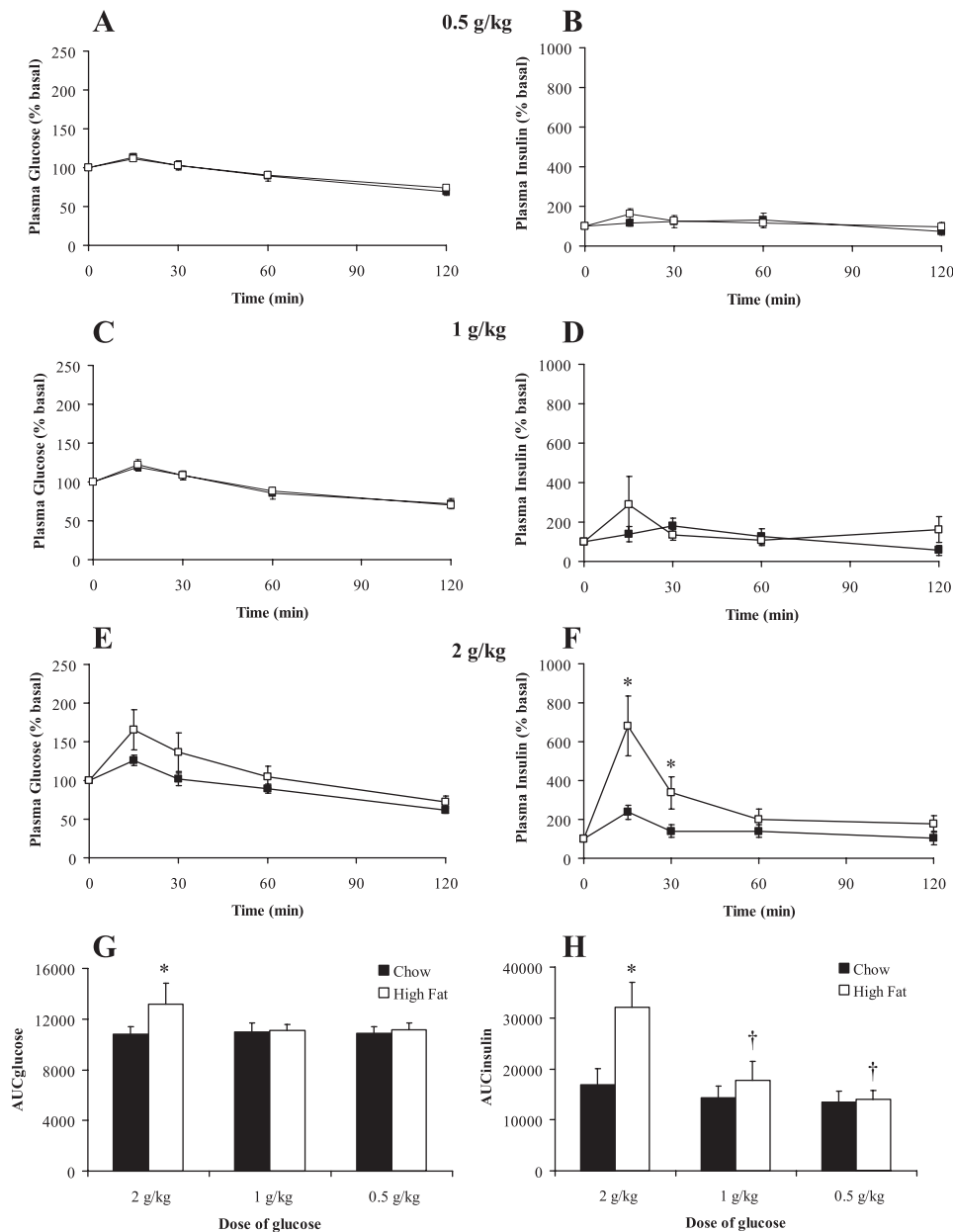


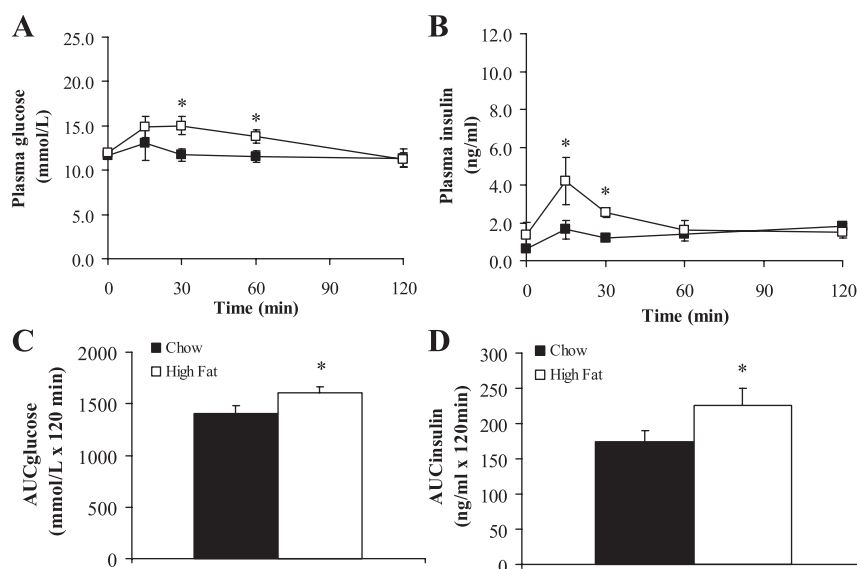
Fig. 5. Plasma glucose and insulin concentrations during the oral glucose administration of 0.5 (A and B), 1.0 (C and D), or 2 g/kg (E and F) glucose following 6 h of fasting in C57BL/6J mice fed a standard chow (■) or high-fat (□) diet for 8 wk. AUC_{glucose} (G) and AUC_{insulin} (H) were calculated using the trapezoidal rule. Results are means $\pm$ SE ($n = 8-10$). * $P < 0.05$, chow vs. high fat; † $P < 0.05$ vs. 2 g/kg, high-fat diet group.

this correlated well with AUC_{insulin} ($r = 0.73$) and $AUC_{\text{insulin}}/AUC_{\text{glucose}}$ ($r = 0.69$) during the glucose tolerance test. Therefore, we suggest that determining HOMA-IR following a 6-h fast may be a useful noninvasive way of determining insulin sensitivity in mice. It is important, however, to note that the euglycemic hyperinsulinemic clamp is considered the “gold standard” method of measuring insulin sensitivity, and our data using 6-h-fasted values to calculate HOMA-IR would need to be validated with the clamp performed on 6-h-fasted mice. Interestingly, a recent study found that there was only a weak correlation between a number of surrogate markers of insulin resistance (including HOMA-IR; $r = -0.44$) and glucose infusion rate during the clamp, but the mice were fasted for 15 h (22). Perhaps a stronger correlation might have been achieved following 6 h of fasting.

The next variable we tested was the route of administration and to determine whether delivering the glucose orally in mice

provides better glucose tolerance data than the intraperitoneal injection. It has previously been shown that there is a 10–20% rate of error with intraperitoneal injections as a result of the needle piercing the lumen of the intestines or the stomach (6, 24), which would obviously impact the rate and amount of glucose appearing in the circulation. Thus one may expect that the intraperitoneal vs. oral glucose delivery would result in different dynamics in the plasma glucose and insulin excursions, which is exactly what we found. There were two striking features of the OGTT: 1) the plasma glucose levels were significantly lower compared with the IPGTT, and 2) the plasma insulin levels increased sharply and peaked at 15 min, whereas during the IPGTT plasma insulin increased at a slower rate, reaching peak levels at 30–60 min. This results in the significant increase at 15 min and AUC_{insulin} in high-fat compared with chow diet during the OGTT, whereas there were trends for increased plasma insulin levels and AUC_{insulin} dur-

Fig. 6. Plasma glucose (A) and insulin (B) concentrations during the OGTT, using a fixed glucose dose of 50 mg/mouse, following 6 h of fasting in C57BL/6J mice fed a standard chow (■) or high-fat (□) diet for 8 wk. AUC_{glucose} (C) and AUC_{insulin} (D) were calculated using the trapezoidal rule. Results are means $\pm$ SE ($n = 7-8$). $*P < 0.05$, chow vs. high-fat.



ing the IPGTT. Similar data in sharp increases in insulin levels were recently obtained by Ahren et al. (3) when intravenous infusion of glucose to match an oral glucose load in chow- and high-fat-fed mice was compared and were attributed to the incretin response. Therefore, we suggest that the OGTT is a more sensitive technique to discern differences in plasma glucose and insulin levels in chow compared with high-fat-fed C57BL/6J mice.

The third variable that we tested was the amount of glucose to administer to the mice. It is evident from a survey of the literature that, in rodent research, there is no consistent amount of glucose given to determine glucose tolerance, with both 1 and 2 g/kg used. In our study, we compared these two doses as well as a third dose of 0.5 g/kg to determine which of these doses would result in the greatest difference in glucose and insulin levels between chow- and high-fat-fed C57BL/6J mice. It is clear that the two lower doses did not result in any difference between chow- and high-fat-fed mice in either glucose or insulin levels. It is only when 2 g/kg glucose was administered that a difference between chow and high fat was robustly evident. These results may not be surprising because the high-fat-fed mice were heavier and received more glucose in absolute terms compared with chow-fed mice. Consequently we conducted a series of experiments in which we delivered a fixed dose of 50 mg of glucose regardless of body weight and found glucose intolerance and higher plasma insulin levels in the high-fat- compared with chow-fed animals. This suggests that under these experimental conditions a fixed dose of glucose can discriminate glucose tolerance. In summary we suggest that, when administered orally, 2 g/kg glucose is an appropriate amount to show a difference between chow- and high-fat-fed C57BL/6J mice.

The final variable tested was state of consciousness. We found that glucose intolerance was present whether the high-fat-fed mice compared with their chow-fed counterparts were anesthetized or conscious. This suggests that, as long as the "control" and "test" mice are treated equally, differences in glucose tolerance can be discerned.

In the last set of experiments, we applied what we had learned to mouse strains that have previously been shown to

have differences in glucose tolerance. Interestingly, we saw little difference in the AUC_{glucose} curve among the six strains tested. However, we did see the expected changes in plasma insulin levels. In particular, as we and others have previously shown, the 129T2 mice had lower insulin levels compared with DBA/2 and C57BL/6J mice (12, 19). Furthermore, DBA/2 mice had higher insulin levels compared with C57BL/6J mice, and we have recently shown that this associated with an increase in the mitochondrial proton pump nicotinamide nucleotide transhydrogenase (7). The highest insulin levels were observed in the NZO mouse, and this is the expected outcome since it is a polygenic model of obesity, glucose intolerance, and insulin resistance (9, 30). These data highlight the importance of measuring plasma insulin levels during the glucose tolerance test.

In conclusion, we have evaluated the glucose tolerance test in the mouse and have determined that administering a 2 g/kg body wt of glucose orally following 6 h of fasting will result in robust differences in plasma glucose and insulin levels in chow- compared with high-fat-fed C57BL/6J mice. For the sake of comparison of the severity of glucose intolerance in different metabolic states and gene manipulations, we suggest that a standard technique for assessing glucose tolerance in mice be agreed upon.

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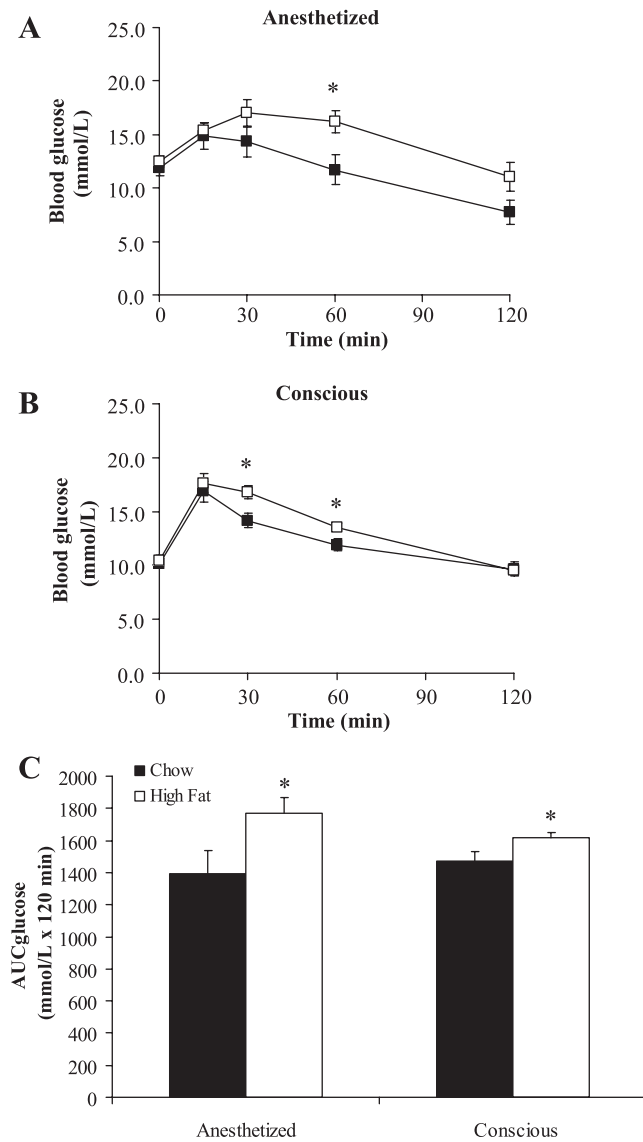


Fig. 7. Blood glucose concentrations during the OGTT in the anesthetized (A) or conscious (B) state, using a fixed glucose dose of 50 mg/mouse, following 6 h of fasting in C57BL/6J mice fed a standard chow (■) or high-fat (□) diet for 8 wk. AUC_{glucose} (C) was calculated using the trapezoidal rule. Results are means $\pm$ SE ($n = 4$). * $P < 0.05$, chow vs. high fat.

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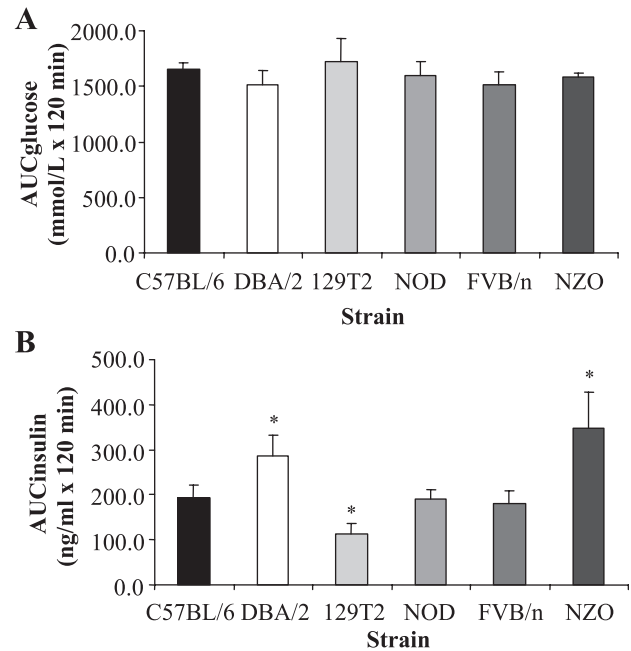


Fig. 8. AUC_{glucose} (A) and AUC_{insulin} (B) curves during the OGTT (2 g/kg) following 6 h of fasting in 10-wk-old C57BL/6J, DBA/2, 129T2, nonobese diabetic (NOD), FVBn, and New Zealand obese (NZO) male mice.

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