Journal of the American Association for Laboratory Animal Science
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Vol 56, No 1
January 2017
Pages 95–97

# Calculation of Glucose Dose for Intraperitoneal Glucose Tolerance Tests in Lean and Obese Mice

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**Glucose tolerance tests are used frequently in nonclinical research with laboratory animals, for example during characterization of obese phenotypes. Despite published standard operating procedures for glucose tolerance tests in rodents, how glucose doses should be calculated when obese and lean animals are compared is not well documented. Typically the glucose dose is calculated as 2 g/kg body weight, regardless of body composition. With this approach, obese mice receive larger glucose doses than do lean animals, potentially leading to overestimation of glucose intolerance in obese animals. In this study, we performed intraperitoneal glucose tolerance tests in mice with diet-induced obesity and their lean controls, with glucose doses based on either the total body weight or the lean body mass of the animals. To determine glucose tolerance, we determined the blood glucose AUC during the glucose tolerance test. We found that the blood glucose AUC was increased significantly in obese mice compared with lean mice by 75% on average when glucose was dosed according to the lean body mass and by 87% when the glucose dose was calculated according to total body weight. Therefore, mice with diet-induced obesity were approximately equally glucose intolerant between the 2 dose-calculation protocols. However, we recommend calculating the glucose dose according to the lean body mass of the mice, because doing so eliminates the concern regarding overdosing of obese animals.**

**Abbreviations:** CI, confidence interval; DIO, diet-induced obesity; GTT, glucose tolerance test; HFD, high-fat diet; IPGTT, intraperitoneal glucose tolerance test; IVGTT, intravenous GTT; OGTT, oral glucose tolerance test.

In nonclinical research with rodents within the field of metabolism and diabetes, the glucose tolerance test (GTT) is frequently used to assess experimental animals. This test applies to characterizing an obese and/or diabetic phenotype, and/or following an experimental treatment intended to improve metabolic parameters. When performing GTT in rodents, common practice is to calculate the glucose dose from the total bodyweight of each animal, irrespective of degree of obesity and body composition. Obese mice typically have increased amounts of adipose tissue but not lean body mass, which is the primary compartment for glucose disposal.[5] If glucose doses are calculated based on the total bodyweight, the lean body mass of an obese mouse is exposed to larger amounts of glucose than that of a lean mouse, and this could influence the result of the GTT. Furthermore it has been shown that both db/db and ob/ob mice, which weighed approximately 100% more than lean age-matched control mice, only had an increased total blood volume of approximately 25%.[8] As the readout of a GTT is the concentration of glucose in the blood, it can be speculated that the blood volume could influence the GTT results. Typically, mice with diet-induced obesity (DIO) are used in experiments when their bodyweight is increased with 20% to 50% compared with lean controls. We hypothesize that their total blood volume at this stage of obesity is only marginally increased compared with lean control mice. A GTT performed with glucose doses calculated based on the total bodyweight could therefore hypothetically overestimate the glucose intolerance of the obese mice, also because their blood volume is approximately similar to that of lean mice. Clarification of these concerns is important for correct design of GTT in obese and lean animals. In this case study, we have explored glucose tolerance in obese and lean mice, with glucose doses calculated from total bodyweight and lean body mass. On the basis of our results, we recommend a method for calculating glucose doses for intraperitoneal GTT in mice.

*Received: 29 Apr 2016. Revision requested: 31 May 2016. Accepted: 13 Jun 2016.*
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## Case Report

The aim of this study was to assess whether glucose doses calculated from the total bodyweight or the lean body mass influence the observed glucose tolerance in DIO-mice and lean control mice.

## Materials and Methods

The study population comprised male C57BL/6NTac mice (Taconic, Ejby, Denmark) purchased at 6 wk of age. All experimental procedures involving animals were performed with permission from the Danish national authority, the Animal Experiments Inspectorate. After arrival, 30 mice were given unrestricted access to a pelleted high-fat diet (HFD; 60 kcal%; RD12492, Research Diets, New Brunswick, NJ), and the 30 mice in the lean control group were fed a standard pelleted rodent chow (Altromin 1324, Brogården, Hørsholm, Denmark). Mice were housed 10 per cage in standard plastic (floor area, 1800 $cm^2$) cages in a ventilated room at 18 to 24 °C, a 12:12-h light:dark cycle, relative humidity of 30% to 70%, and 8 to 15 air changes hourly.

The glucose tolerance of the mice was examined after HFD feeding for 8, 10, and 11 wk (10 obese and 10 lean mice were examined at each time point, except for the 10-wk point, when one lean mouse was excluded due to bite wounds). At each time point, each mouse underwent quantitative magnetic resonance analysis (EchoMRI, Houston, TX) to estimate body composition (that is, total lean body mass and total fat mass). Glucose tolerance was assessed by intraperitoneal GTT (IPGTT) in unan-

esthetized mice. Each mouse underwent 2 IPGTT—one in which the glucose dose was calculated on the basis of the total body weight and the other in which the glucose dose was calculated according to total lean mass. The GTT were performed during a single testing session each day on 2 sequential days, when lean and obese mice were allocated randomly into 2 subgroups. On the first test day, half of the lean and obese mice received the glucose dose calculated according to total body weight and the other half the dose determined based on lean body mass; on the second test day, each group received the other dose. The mice were fasted for 6 h prior to the GTT (food removed at 0700). Blood glucose was measured according to the manufacturer's instructions (BIOSEN glucose analyzer, EKF Diagnostics, Barleben, Germany) in 5-µL samples collected from a small incision made at the tip of the tail immediately before treatment and at 15, 30, 60, and 120 min after intraperitoneal injection of glucose, and the AUC describing blood glucose levels in each mouse during IPGTT was calculated (Prism 6.0, GraphPad Software, San Diego, CA). Plasma concentration of endogenous insulin was not measured during the GTT.

After completion of IPGTT on the second test day, the mice were injected with 100 µL isotonic saline containing Evans blue dye (total dose, 90 µg; 0.9 mg/mL; Sigma-Aldrich Denmark A/S, Copenhagen, Denmark) through a tail vein; 10 min later, a blood sample was collected from the tongue vein, for assessment of Hct and the plasma concentration of Evans blue for estimating the total blood volume as described previously,[7] except that volumes were adjusted for mice. Because this method is very sensitive to inaccuracies in the administration of Evans blue, blood volume was only estimated in mice that successfully received the entire injection (13 DIO mice and 19 lean mice).

Statistical analysis was done by using Prism 6.0 (measured blood glucose data) and JMP 10.0.2 (all other data; SAS Institute, Cary, NC). A $P$ value less than 0.05 was considered statistically significant. Blood glucose values at each time point during IPGTT were compared between lean and obese mice by using an unpaired 2-sided $t$ test, with Sidak correction for multiple pairwise comparisons. Furthermore, the mean ratio between obese and lean mice and the 95% confidence interval (CI) of this ratio were determined for all parameters examined (body weight, lean body mass, fat mass, blood volume, glucose dose, blood glucose AUC) by using logarithmically transformed data. These results were then back-transformed so that differences and 95% CI could be expressed as ratios. Lean body mass was included as a covariate when the total blood volume was compared between obese and lean mice.

## Results

Results from GTT in the mice after feeding standard chow or HFD for 8, 10, and 11 wk (Figure 1) were comparable; therefore, data from all mice in each diet group were combined. Exposure to HFD for 8 to 11 wk significantly ($P < 0.0001$) increased the total body weight of the mice by an average of 24% (Table 1). This effect was driven by the large relative increase in the total fat mass (280% on average), whereas the total lean mass showed only a nonsignificant trend toward a larger total lean mass (3%) in the obese mice. In addition, the total blood volume was comparable between the obese and lean mice, with a nonsignificant trend toward a 6% larger blood volume in obese mice. Glucose doses calculated from the total body weight were on average 24% higher in the obese mice than in the lean mice, and the mean blood glucose AUC during IPGTT was 87% higher in the obese mice than the lean mice ($P < 0.001$). When the glucose doses were based on the lean body mass of the animals, the doses were comparable between lean and obese mice, that is, on average only 3% higher in obese animals. Despite this similarity, the obese mice remained overtly glucose intolerant: their mean AUC was 75% higher than that of lean control mice ($P < 0.0001$).

## Discussion

The key findings in this case study are that DIO led to a gain in fat mass, whereas lean body mass and total blood volume were only marginally increased in DIO mice compared with lean mice. Furthermore, DIO mice were glucose intolerant according to IPGTT, regardless of whether the glucose dose was calculated on the basis of total body weight or lean body mass. We believe that finding is important and demonstrates that the glucose intolerance of DIO mice in our study was not overestimated when glucose was dosed according to total body weight, even though the DIO mice received a larger glucose dose than did lean mice in this situation. In fact, the AUC describing blood glucose concentration during IPGTT was increased to a similar degree in DIO mice compared with lean mice in our study, regardless of how the glucose doses were calculated (note the overlapping CI in Table 1).

Several previous studies prompted concerns regarding the effect of the method for calculation of the glucose dose on the results of GTT in rodents.[2,3,5,6] However, only few studies have addressed the question. One group[2] compared different doses of glucose administered during IPGTT and oral GTT (OGTT) in combination with different fasting periods; they also assessed a fixed dose of glucose (50 mg per mouse) during OGTT. The authors[2] reported that a glucose dose of 2 g/kg total body weight resulted in the largest window for demonstration of glucose intolerance, and the mean AUC describing blood glucose was 27% and 47% increased during the IPGTT and OGTT, respectively, in DIO mice. In addition, at the fixed dose of 50 mg per mouse, the mean AUC during the OGTT was significantly increased in DIO mice, but only by 15% to 25%. Consequently, the authors recommended a glucose dose of 2 g/kg total body weight for OGTT.[2] Another study[6] explored glucose tolerance in DIO and lean mice by performing intravenous GTT (IVGTT) with glucose doses calculated according to total bodyweight and lean body mass and as a fixed dose that was based on the average body weight of all study animals. These dosing regimens resulted in blood glucose AUC values during the IVGTT that were an average of 110%, 78%, and 118% greater, respectively, in the obese mice than the lean controls, which means that all three dosing regimens induced glucose intolerance. Dosing glucose according to the lean body mass appeared to result in a smaller intergroup difference in AUC than did the 2 other dosing regimens.[6] However, note that the dose of glucose used during the fixed dosing regimen in the cited study[6] was approximately 55% higher than those calculated relative to lean body mass and comparable to those administered according to total body weight to the DIO mice. This fact might also explain why the AUC difference between lean and DIO mice appeared smallest when doses were calculated from the lean body mass. Furthermore, evaluation of β-cell function during the IVGTT was an additional aim of the previous study,[6] and they recommended the fixed-dose approach for IVGTT.

To our knowledge, the current study is the first in which different glucose dosing regimens have been tested in IPGTT. It might be argued that OGTT is preferable to IPGTT for the assessment of glucose intolerance, due to the lack of the incretin response when glucose is administered intraperitoneally.[3,5] However, diabetes and DIO both influence the absorption of glucose from the intestine,[1] and chronic exposure to an energy-dense diet

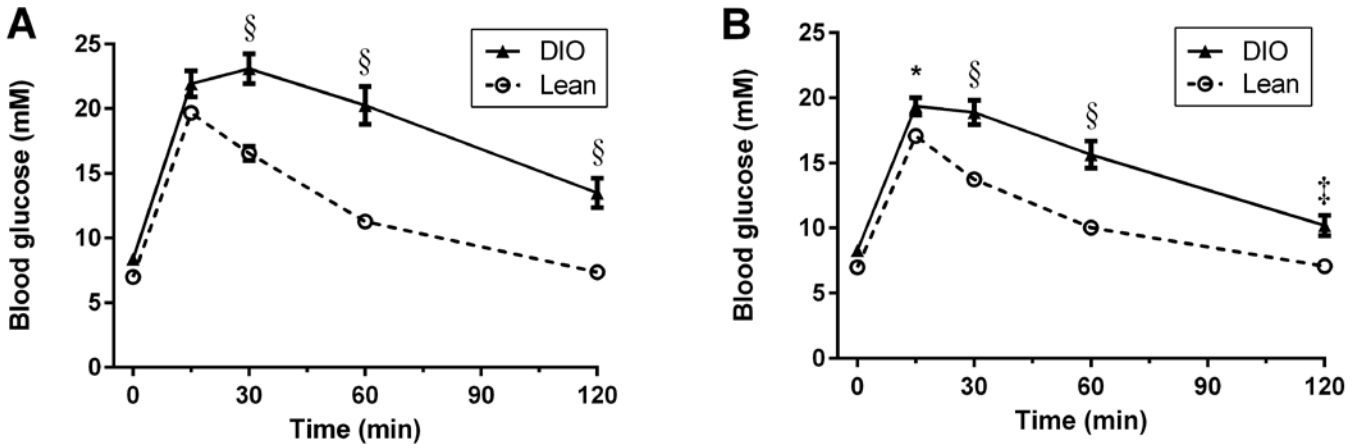


**Figure 1.** Results of GTT (blood glucose concentration, mean [error bars, SEM]) in mice with diet-induced obesity (DIO; $n = 30$) and their lean controls ($n = 29$) when the intraperitoneal glucose dose was calculated according to (A) total body weight or (B) lean body mass. Values that differ significantly (*, $P < 0.05$; ‡, $P < 0.001$; §, $P < 0.0001$) are indicated.

**Table 1.** Parameters associated with glucose tolerance testing in obese and lean mice

| | Obese | Lean | Ratio (95% CI) |
|---|---|---|---|
| Body weight (g) | 39.6 ± 1.0 | 31.8 ± 0.6 | 1.24 (1.16–1.31) |
| Lean body mass (g) | 26.9 ± 0.4 | 26.2 ± 0.5 | 1.03 (0.98–1.08) |
| Fat mass (g) | 9.7 ± 1.0 | 2.1 ± 0.2 | 3.8 (2.7–5.2) |
| Blood volume (mL) | 3.5 ± 0.2 | 3.2 ± 0.1 | 1.06 (0.97–1.17) |
| Intraperitoneal glucose dose$_{\text{total body weight}}$ (mg) | 79.5 ± 2.0 | 63.6 ± 1.2 | 1.24 (1.17–32.0) |
| IPGTT$_{\text{total body weight}}$ AUC (mM × min) | 1222.4 ± 93.6 | 612.1 ± 37.2 | 1.87 (1.55–2.24) |
| Intraperitoneal glucose dose$_{\text{lean body mass}}$ (mg) | 53.8 ± 0.7 | 52.5 ± 1.1 | 1.03 (0.98–1.08) |
| IPGTT$_{\text{lean body mass}}$ AUC (mM × min) | 795.5 ± 57.4 | 450.6 ± 27.3 | 1.75 (1.50–2.05) |

CI, confidence interval
Data are given as mean ± SEM.

influences the size of the intestine.[4] Therefore, several factors can complicate the interpretation of OGTT performed in mice with different metabolic phenotypes or fed different diets. Furthermore, IPGTT is less technically challenging than is IVGTT, for which blood sampling is more frequent and catheterization is often necessary. By far IPGTT is the most frequently applied type of GTT in mice;[2] therefore further exploring the influence of glucose doses on IPGTT is important.

Here we have demonstrated that, according to results from IPGTT, DIO mice fed HFD for 8 to 11 wk appeared to be glucose intolerant when compared with age-matched lean control mice, regardless of whether glucose was dosed relative to the total body weight or lean body mass. Although these findings show that the glucose intolerance was not overestimated when glucose was dosed per total body weight, we recommend that the calculation of glucose doses for IPGTT as 2 g/kg of lean body mass in both the lean and obese animals. Of the various approaches we tested, this approach was physiologically the most correct approach, because it prevents over-dosing of the obese mice with glucose, and this potential concern is then eliminated. If assessing the lean body mass of the mice in a given study is impossible, the glucose dose alternatively could be based on the average total body weight of the lean mice (that is, a fixed dose given to all mice), because this parameter likely will be only slightly higher than the average lean body mass of lean as well as obese mice.

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