Comparison of Glucose Administration Routes to Determine Glucose Tolerance in Old and Young Mice

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ABSTRACT

Background: It has been observed that old mice have a better tolerance to glucose than young after an intraperitoneal injection of glucose. We hypothesized that an intraperitoneal injection of glucose overestimates glucose tolerance in old mice.

Methods: We assessed differences in glucose tolerance outcomes in 2- (young, n=23) and 23- to 27-month-old (old, n=23) male C57BL/6J mice after an intraperitoneal (IP) or intravenous (IV) injection, or oral gavage (OG) of glucose.

Results: The area under the curve (AUC) of the changes of blood glucose concentration over 2 hours after glucose administration was lower in old than young animals (p<0.001). The AUC was higher after IV than either IP or OG (p<0.001). However, normalized to peak glucose concentration, the time course was similar in young and old animals, and was – except for an earlier peak in IV than OG (p=0.013) – independent of route of administration.

Conclusion: We suggest that 1) to determine glucose tolerance the time course of changes in glucose concentration rather than the AUC, which is significantly affected by excursion magnitude, is preferable; 2) glucose tolerance can be measured equally well with IP, IV and OG; 3) there is no significant age-related difference in glucose tolerance in mice.

Keywords: glucose tolerance test, glucose uptake, aging, glucose intolerance, mice, diabetes.

INTRODUCTION

Glucose tolerance has been defined as the ability to dispose of a glucose load, where glucose intolerance is the impaired disposal of glucose after a challenge is referred to as glucose tolerance test (Ahrén, 2012). Glucose intolerance is the underlying cause of many metabolic disorders including diabetes (Stout, 1994) and is commonly seen in older adults (Basu et al., 2003; Shimokata et al., 1991; Stout, 1994). Glucose intolerance is a reflection of insulin resistance and/or beta cell dysfunction (Gunasekaran & Gannon, 2011; Li et al., 2014).

Murine models are widely used to study the development of metabolic disorders like diabetes, obesity, and cardiovascular disease during aging. The glucose tolerance test (GTT) is used routinely to determine tolerance to glucose in mouse models, particularly C57BL6J (Ayala et al., 2010). This test is usually performed by assessing the changes in blood glucose concentrations over time in response to an intraperitoneal bolus injection of glucose after overnight fasting. In contrast to the humans, some studies have reported better, rather than poorer, glucose tolerance in old (even as old as 28 months) compared to young mice (De Leon et al., 2018; Oh et al., 2016; Reynolds et al., 2019). This has been explained by adaptations in old age

such as an increase in the size of beta cells (De Leon et al., 2018) and a rise in number of calcium receptors (Oh et al., 2016), which lead to better glucose metabolism. However, an intraperitoneal (IP) injection may be biased by possible age-related differences in glucose uptake from the peritoneum into the circulation due for instance to accumulation of adipose tissue in older mice that will cause a slower release of glucose. In line with this, Konrad et al., 2007 observed that after an intra-abdominal fat transplant in mice, glucose tolerance to an IP challenge was improved rather than impaired. Earlier studies have also reported a 10–20% margin of error with IP injections for the rate of glucose uptake and raised questions about the efficacy of IP to measure glucose tolerance (Arioli & Rossi, 1970; Benjamin & Casper, 1966; Miner et al., 1969), but to our knowledge had not been further explored. In addition, it has been reported that oral administration of glucose was more sensitive to detect changes in glucose tolerance than intraperitoneal administration of glucose (Andrikopoulos et al., 2008). However, even with the oral glucose administration inconsistencies in results a lack of reproducibility of GTT has been reported in humans (Nelson, 1988), so care must be taken while interpreting the results. Based on these observations, we suggest that to circumvent potential age-related differences in the rate of absorption of glucose into the circulation, the best way to assess glucose tolerance is direct injection into the circulation. Indeed, it has been observed that 56% of pregnant women suggested to have abnormal glucose tolerance after an oral GTT had normal glucose tolerance when assessed with a venous GTT (Benjamin & Casper, 1966). However, there are no systematic comparisons between the measured glucose tolerance derived from intraperitoneal, intravenous or oral administration of glucose in young and old mice.

We hypothesized that an oral gavage and intraperitoneal injection of glucose overestimate glucose tolerance in old but not young mice. Therefore, the aim of this study was to assess the differences in glucose tolerance in young and old mice after an intraperitoneal (IP) or intravenous (IV) injection, or oral gavage (OG) of glucose.

METHODS

All experiments were approved by the ethics committee of the Lithuanian Republic Alimentary and Veterinary Public Office (#G2-90 in 2018).

Male C57BL/6J mice were bred internally from an original population purchased from The Jackson Laboratory (USA). At least one month prior to the experiment, they were housed individually at 20–22 °C in a 12h light/dark cycle at the animal research facility at the Lithuanian Sports University. Animals had free access to water and standard chow (65/66% carbohydrate, 21/24% protein and 6/11% fat of the total kCal intake; UAB Joniškio grūdai, Lithuania/Altromin, Lage, Germany). Two (young, n=23) and 23 to 27-month-old (old, n=23) male mice were used for this study.

After 16 hours overnight fasting, the body mass was measured on a scale (440-45N, Kern, Germany). Then the mice received an intraperitoneal injection (IP: young n=7; old n=6), an intravenous injection via the tail vein (IV: young n=7; old n=6) or oral gavage (OG: young n=9; old n=11) of glucose (2 g glucose/kg body mass). Injections were performed using a 29 G needle, while OG was performed with a custom-made feeding tube. The glucose solution (20%) was at room temperature and delivered via IP, IV or OG within one minute. A glucometer (Glucocard X-mini plus, Japan) was used to measure blood glucose from a drop of blood taken from an incision made in the tail vein at baseline (0 min) and at 15, 30, 60, 90 and 120 min after the glucose administration. The animals were not anaesthetized during injection, gavage and the measurements. The IV injection was performed carefully to ensure that all the glucose solution was administered. During the experiment, the mice were kept individually, had access to water and were able to roam freely in their cage. Prism 7.0 software was used to calculate the area under the glucose – time curve (AUC).

Statistical analyses

Data are presented as mean ± SEM. An ANOVA was used to test for differences in body mass and area under the curve of glucose, with age and route of injection, where appropriate, as factors. To determine the changes in blood glucose over 120 mins, a repeated-measures ANOVA with time as within factor, and age and route of injection as between factors was used. If significant interactions or time effects were found, post-hoc Bonferroni-corrected t-tests were performed to detect differences between time points and ANOVAs for young and old animals were performed separately. There were no significant time * age * group interactions. Effects were considered significant at $p < 0.05$. All calculations were performed using IBM SPSS Version 23.

RESULTS

The body mass of old (31.7 0.9 g) was larger than that of the young (21.2 ± 0.4 g) animals (p<0.001).

Rate of glucose uptake

Figure 1 shows the time-course of the changes in the blood glucose concentration up to 2 hours after administration of glucose (2 g glucose/kg body mass). At baseline, the glucose concentration was similar in young and old animals (p=0.774). There was no significant age * route of administration interaction (p=0.191), indicating that any differences due to the route of administration were similar in young and old mice.

There were, however, significant effects of time (p<0.001), age (p<0.001) and route of glucose injection (p<0.001), and significant time * age (p=0.001) and time * route of glucose administration interactions (p<0.001). The glucose concentration at any time point was higher after an IV than IP injection or OG (p<0.001), with no significant differences between IP and OG (Fig. 1A). At 15–120 min the glucose concentration for each route of administration was higher in young than old animals (p≤0.001) and at 120 min it was in both young and old animals still higher than at baseline (p≤0.026; Fig. 1A).

Figure 1. **A) Glucose concentration (mM) and B) glucose concentration normalized to the peak glucose concentration during the test from 0 to 120 minutes (mins) in young (Y) and old (O) mice after administration of glucose intravenously (IV) via the tail vein, intraperitoneally (IP) or via oral gavage (OG). 1, 2, 3, 4, 5: significantly different from 0, 15, 30, 60 and 90 mins respectively at p≤0.049, irrespective of route of administration of glucose (the numbers 1 to 5 at the upper side of the graph refer to the young group). Age: indicates that at any timepoint (except time 0 min) old values were lower than corresponding young values at p≤0.001. Group indicates that in all cases the values of the IV route were higher than those in the IP and OG group at p<0.001; OG: OG < IV, IP at p=0.011. In panel B) IV>OG at p=0.013; IP>IV,OG at p≤0.028. Data is presented as mean ± SEM.**
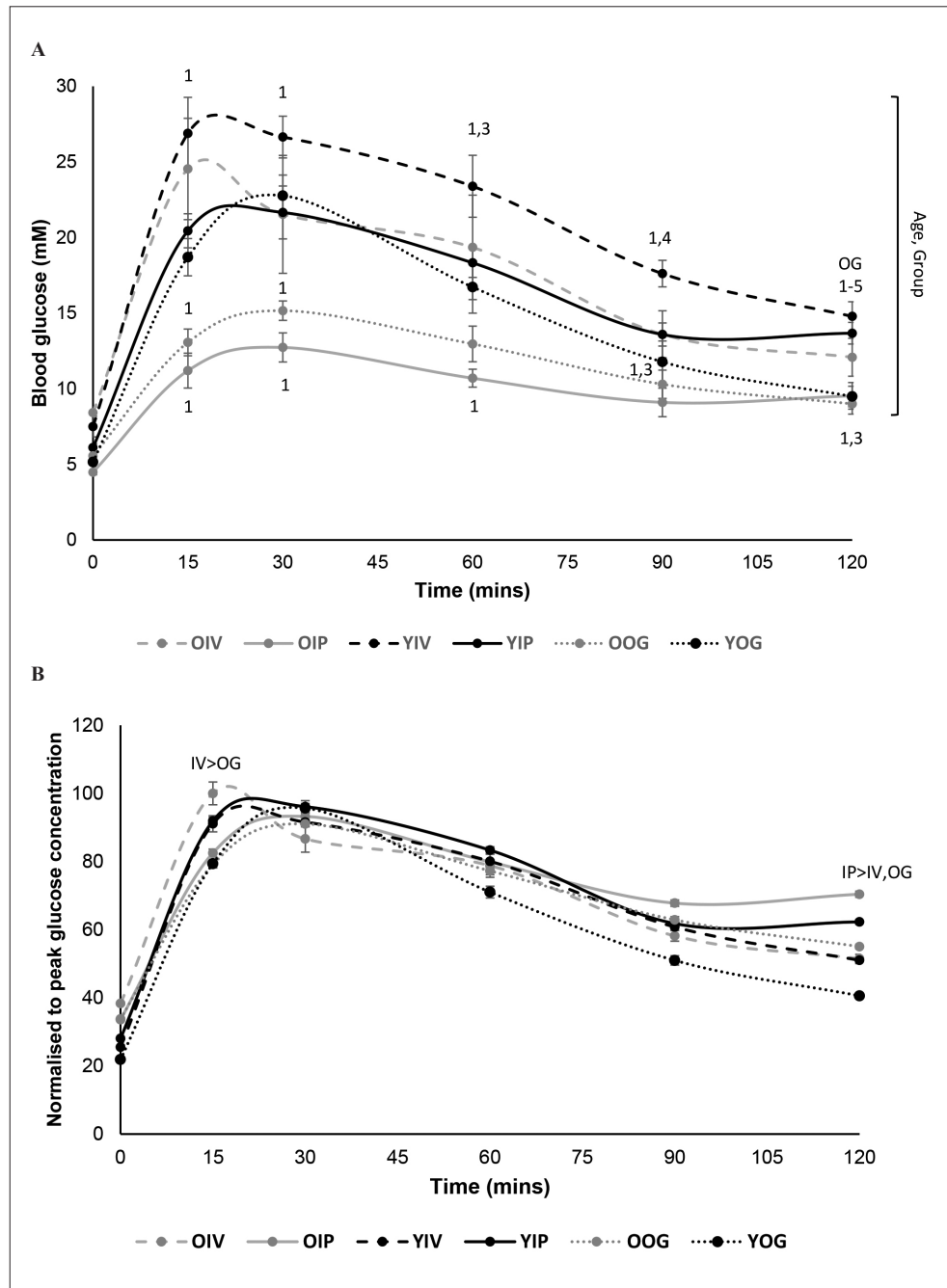
To get a better insight in the time * route of administration interaction, we normalized the data to the highest measured concentration during a test (Fig. 1B). For the normalized data, there were no significant main effects of age or route of administration, but the time * route of administration interaction (p=0.019) was reflected by a higher glucose concentration at 15 min in IV than OG (p=0.013) and at 120 min in IP than in IV and OG (p≤0.028). This reflects that IV resulted in an earlier peak in glucose concentration than OG.

The area under the curve (AUC), calculated from blood glucose concentration measurements at 0, 15, 30, 60, 90 and 120 minutes, was lower after an OG or an IP injection compared to an IV injection of glucose in both young and old animals (p<0.001). Old animals had a lower AUC than the young (p<0.001), irrespective of the method by which glucose was administered (route * age interaction: p=0.296) (Fig. 2A). A stepwise regression indicated that the AUC was positively related to the highest measured glucose concentration during the test (R^2_{adj}=0.774; p<0.001; Fig. 2B), with a small

Figure 2. **A) Area under the curve in arbitrary units calculated from blood glucose concentrations (mM) at 0, 15, 30, 60, 90 and 120 minutes after administration of bolus glucose intravenously (IV) via the tail vein, intraperitoneally (IP) or via oral gavage (OG) in young (Y) and old (O) mice. Age: indicates that at any timepoint (except time 0 min) old values were lower than corresponding young values at p<0.001; IV: indicates significantly different from IV at p<0.001; Data is presented as mean, min, max. B) Correlation between area under the curve and peak glucose concentration during the test. R^2_{adj}=0.774; p<0.001.**

contribution of the route of administration that increased the R^2_{adj} by 0.014 (R^2_{adj}=0.788; p<0.001), but no significant effect of body mass.
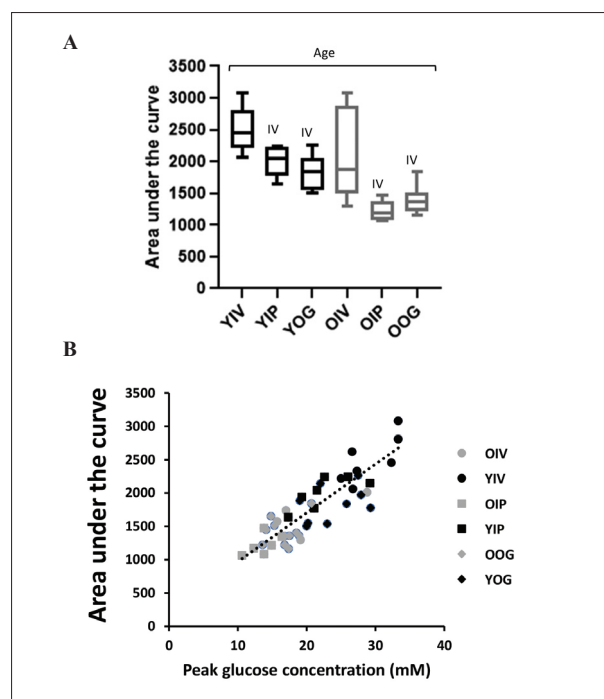
DISCUSSION

The main observation of the present study is that the area under the glucose-time curve (AUC) was positively related to the peak glucose concentration during the glucose tolerance test, irrespective of administration route. The impact of the peak glucose concentration can be accommodated by normalizing the glucose concentrations to the peak concentration during the test and this showed that oral gavage, an intraperitoneal or intravenous injection of glucose can all be used to measure glucose tolerance in mice. Secondly, although the AUC was lower in old than young mice, the time course of changes in glucose concentration was similar, indicating that there was no significant difference in glucose tolerance between young and old mice.

The baseline glucose levels of the animals in our study were similar to that seen in previous research (Ayala et al., 2006). Our data shows that after routine overnight fasting, both young and old animals had similar baseline glucose concentration. Therefore, any age-related or route of injection of glucose-related differences in glucose tolerance cannot be attributed to different starting glucose concentrations.

Similar to previous studies (De Leon et al., 2018; Oh et al., 2016), we observed that the AUC was lower in old than young mice, no matter the mode of administration. Two possible adaptations were described to explain this phenomenon – an increase in size of beta cells which led to an increase in insulin secretion (De Leon et al., 2018) and/or an increase in the number of calcium receptors which increased the metabolism of glucose (Oh et al., 2016). The lower AUC observed in old mice may be a result of visceral adipose tissue accumulation slowing down the glucose uptake after IP injection. This phenomenon has been observed previously in mice that underwent an intra-abdominal fat transplant and showed a better rather than worse tolerance to an IP injection of glucose (Konrad et al., 2007). In further support of such an effect, it has been shown that the rate of glucose appearance was slower in obese than lean mice (Small et al., 2022). We did, however, see no correlation between body mass (or age) with the AUC, but we did not

determine the amount of visceral fat, or whole-body fat percentage in our mice. Whatever the potential impact of visceral fat on glucose appearance in the blood in old animals, the degree of adiposity does not directly affect the glucose uptake after oral gavage (Small et al., 2022), as also shown here, or intravenous injection.

We found that an intraperitoneal injection or oral administration of glucose led to a significantly smaller AUC than that seen after an intravenous injection. Thus, the glucose tolerance in old mice in previous studies using intraperitoneal injections of glucose (De Leon et al., 2018; Oh et al., 2016) may well have been overestimated. In addition to the smaller AUC after oral or intraperitoneal administration than intravenous injection of glucose, the peak glucose concentration occurred 15 min after an intravenous and 30 min after an intraperitoneal injection or oral administration of glucose. This suggests that an intraperitoneal or oral administration led to a slower uptake of glucose in the blood contributing to an apparently better glucose tolerance than after an intravenous injection. An IV injection ensures that glucose directly enters the blood and, one may argue, is therefore a more robust method to measure the ability to maintain glucose homeostasis in the circulation than that derived from an intraperitoneal injection or oral gavage, where in the latter some of the glucose may also sequestered into the liver via the portal circulation before entering the systemic circulation.

While the AUC is often used as a measure of glucose tolerance (De Leon et al., 2018; Konrad et al., 2007; Oh et al., 2016; Small et al., 2022) we found here that it is strongly related to the peak glucose concentration during the test (R^2_{adj}=0.774; P<0.001), irrespective of the route of administration, body mass, or age of the animal. In other words, a lower peak glucose concentration results in a lower AUC. In an elegant study it has been shown that oral gavage resulted in a lower release of exogenous glucose into the blood than that seen after an intraperitoneal injection which was accompanied by a lower AUC, but a similar time course of changes in the glucose concentration (Small et al., 2022). This then reflects that if the glucose challenge is larger (higher peak) the responses that remove glucose from the blood are also larger; in other words, a higher AUC does not (necessarily) indicate a lower glucose tolerance. To assess glucose tolerance, we suggest it is perhaps more appropriate to look at the shape of the curve, where a prolonged elevated glucose concentration reflects an inadequate response and hence is indicative for a lower glucose tolerance. When we compared the time course of the different routes of administration, we found that 1) except for an earlier peak after an intravenous injection, each route of administration can be used to determine glucose tolerance in mice, and 2) that young and old mice had a similar glucose tolerance.

In conclusion, since a glucose tolerance test is widely used to study glucose metabolism in mice, it is important to choose the right route of glucose administration to get reliable results. Our data suggest that oral gavage, or intraperitoneal or intravenous administration of glucose can all be used to measure glucose tolerance in both old and young mice. Secondly, the glucose tolerance was similar in young (2 months old) and old (23 to 27 months old) mice.

Future work

Whilst our data have yielded interesting and potentially impactful results, we suggest some areas that could be explored to further substantiate our findings. The changes in adipose tissue distribution and insulin function with aging are well documented and it would be interesting to measure (i) fat mass – visceral fat in particular to see how this affects the rate of glucose uptake after an IP injection or oral gavage; (ii) fasting insulin and insulin responses after the glucose challenge to test the response after IP, OG and IV glucose injections; and iii) test whether indeed the time course of return to normal glucose concentrations is prolonged in mice that are known to be glucose intolerant, such as a diabetic or obese mouse model.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author contributions

All authors contributed equally to this manuscript.

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