Metabolic Responses in a Model of Insulin Resistance: Comparison Between Oral Glucose and Meal Tolerance Tests

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The purpose of this investigation was to compare the benefits of a meal tolerance test (MTT) against those of an oral glucose tolerance test (OGTT) in one of the most commonly used models of insulin resistance, the Zucker fatty rat. Comparison of these two oral challenges will facilitate determination of the most effective means of inducing both glucose and insulin responses in this particular model and allow for possible therapeutic benefits to be examined more effectively. Eight-week-old Zucker fatty rats (n = 7 or 8) were used to perform either an OGTT or a MTT following an overnight fast. The OGTT contained a final amount of carbohydrate (CHO) of 1.2 g/kg body weight (BW). The MTT (commercially available liquid meal), in addition to having fat and protein, included a final amount of available CHO and volume to match the OGTT. A saline-treated group served as control. A greater glucose excursion was observed following the OGTT compared to the MTT. The maximal change in glucose from baseline was 140 ± 10 mg/dL (a 2.1-fold rise) for the OGTT compared to 86.3 ± 6.1 mg/dL (a 1.7-fold rise) for the MTT ($P < .05$). The MTT induced a greater change from baseline in insulin response compared to the OGTT (7.5 ± 1.1 *v* 3.9 ± 0.5 ng/mL, MTT *v* OGTT, respectively; $P < .05$). The saline challenge induced only minimal glucose and insulin responses in comparison to the other treatments. These results suggest that, in a model of insulin resistance, the MTT is a more potent insulin stimulator than glucose alone. A mixed meal, such as a MTT, provides a complete nutrient challenge (CHO, fat, and protein) that will induce both glucose and insulin responses, enabling a better capacity to detect differences in one of the most often used models of insulin resistance, the Zucker fatty rat.

THE GENETICALLY obese Zucker fatty rat is one of the most commonly used models of insulin resistance and is characterized by mild hyperglycemia, abnormal glucose tolerance, hyperinsulinemia, and insulin resistance of the liver, skeletal muscle, and fat.[1-3] A mutation of the leptin receptor gene is responsible for the diseased state in this animal.[4,5] More precisely, the Zucker fatty rat phenotype is due to a missense mutation of a Gln residue to a Pro residue at position 269 of the leptin receptor.[6] The progressive development of glucose intolerance and hyperinsulinemia in this particular model has been characterized by using an oral glucose tolerance test (OGTT) as a challenge to induce glucose and insulin responses.[7] However, a complete nutrient challenge incorporating carbohydrate (CHO), fat, and protein has not been characterized in this model. Amplified insulin and reduced glucose responses with a meal tolerance test (MTT) are predicted, due primarily to the fat contained in a mixed meal and to the well-established slowed gastric emptying when compared to glucose alone.[8-10] In addition, amino acids derived from proteins contained in a mixed liquid meal, particularly arginine, are potent insulinotropic agents and could also contribute to the greater insulin response. Human experiments have demonstrated that arginine stimulates an insulin response in healthy subjects.[11] Finally, Sennitt et al reported the effects of a liquid, milk-based meal (with no comparison to an OGTT) on glucose but not on insulin responses in the non–insulin-resistant Sprague Dawley rat.[12] There has been no published comparison, to date, of the effects of an OGTT and of a MTT, both by oral administration and of equivalent CHO loads, on glucose and insulin responses in the Zucker fatty rat. Recently, the effects of an intravenous glucose tolerance test (IVGTT), MTT, and intraperitoneal glucose tolerance test (IPGTT) were reported in the JCR:LA-corpulent rat.[13] However, the different challenges used different routes of administration and the CHO loads were not matched.

In this study, we compared the effects of either challenge (MTT *v* OGTT) in 8-to 9-week-old Zucker fatty rats in order to determine which challenge would have a better capacity to detect differences in glucose and insulin responses in one of the most commonly used models of insulin resistance. The purpose of these experiments was to determine if the MTT provides a simple, reproducible, oral, complete nutrient challenge that can be used as a tool to assess glucose and insulin excursions in a commonly used model of insulin resistance, the Zucker fatty rat.

MATERIALS AND METHODS

Animals Used

Animals were treated in conformity with the Abbott Laboratories Institutional Animal Care and Use Committee (IACUC) guidelines.

Genetically obese Zucker fatty rats (n = 7 or 8/treatment) obtained from Harlan (Madison, WI) were housed in groups of 4 per cage, on a 12-hour/12-hour light/dark cycle and given free access to water and rat chow (Teklad Rodent Diet 8640; Harlan). Rats were acclimatized for 1 week prior to study. It is noteworthy that we carefully handled the animals in order to minimize stress during gavaging. The rise in plasma glucose was minimal with the saline control challenge compared to the OGTT or the MTT groups. Gavaging is a commonly used procedure for the administration of glucose and meal challenges in the study of metabolic parameters.[7,12,14,15]

Oral Glucose and Meal Tolerance Tests

All experiments were performed after an overnight fast at approximately 8 AM. The 3 different groups (saline, MTT, OGTT) were

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randomized based on their average body weights (BW) and fasting glucose levels.

The OGTT (45% solution wt/vol; Abbott Laboratories, North Chicago, IL) was administered by oral gavage at a dose of 1.2 g CHO/kg BW. The MTT, Vanilla Ensure Plus (50 g total CHO; 28%, 58%, and 14% of calories from fat, CHO, and protein, respectively) (Ross Products Division, Abbott Laboratories, Columbus, OH) was administered by oral gavage at a dose of 1.2 g CHO/kg BW (133 μg arginine/kg BW provided at this dose). Finally, as a control, saline was administered by oral gavage (volume administered was matched with that of the OGTT and the MTT groups for volume equivalence across all treatments). Blood samples (~50 to 60 μL) were obtained, by tail snip at 0, 15, 30, 60, and 120 minutes following the different challenges for determination of plasma glucose and insulin. Blood samples for insulin were centrifuged and plasma was stored at -80°C.

Glucose and Insulin Determinations

Plasma glucose was determined immediately on fresh samples by using the Medisense Precision G glucose testing system (Medisense Products, Abbott Laboratories, Bedford, MA). Plasma insulin was measured by a rat insulin enzyme-linked immunossay (ELISA) kit (Alpco Diagnostics, Winham, NH). Incremental area under the plasma glucose or insulin response curves (AUC) was calculated according to the method of Wolever and Jenkins.[16]

Statistical Analysis

Results are given as the mean ± SEM for the indicated number of rats. A 1-way analysis of variance followed by a Tukey-Kramer multiple comparisons test was used. *P* values of .05 (2-tailed) and lower were considered statistically significant.

RESULTS

Body weights were 342 ± 10 (saline), 319 ± 9 (MTT), and 345 ± 6 g (OGTT) ($P > .05$, not significant [NS]) and fasting glucose levels were 118 ± 3 (saline), 121 ± 5 (MTT) and 123 ± 6 mg/dL (OGTT) (n = 7 or 8, $P > .05$, NS).

Figure 1 illustrates plasma glucose levels following a MTT, an OGTT, or a saline control challenge in Zucker fatty rats (n = 7 or 8). Plasma glucose changes from baseline were 86.3 ± 6.1 (MTT) versus 115.2 ± 3.7 (OGTT), 78.1 ± 5.6 (MTT) versus 139.6 ± 10 (OGTT), 72.3 ± 3.9 (MTT) versus 130.4 ± 9.5 (OGTT) and 32.5 ± 4 (MTT) versus 35.2 ± 2.7 mg/dL (OGTT) at 15, 30, 60, and 120 minutes, respectively ($P < .001$, MTT *v* OGTT) (Fig 2). The saline control challenge induced an increase in glucose levels, which was substantially less in comparison to the other treatments (2.7 to 3.7 times lower increase in glucose compared to the MTT or OGTT) (Fig 2). Finally, the OGTT was more potent in elevating plasma glucose, even after 60 minutes (change from baseline, 130.4 ± 9.4 mg/dL) compared to the MTT (change from baseline, 72.3 ± 3.9 mg/dL) and the saline control treatment (change from baseline, 50.6 ± 6.3 mg/dL) (n = 7 or 8, $P < .001$, OGTT *v* MTT or saline) (Fig 2).

Fasting insulin levels were 7.2 ± 0.7 (n = 8, saline), 6.8 ± 0.3 (n = 8, MTT), and 8.7 ± 1 ng/mL (n = 7, OGTT). Figure 3 illustrates plasma insulin levels following either a MTT, an OGTT, or a saline control challenge (n = 7 or 8). Changes from baseline were 7.5 ± 1.1 versus 3.9 ± 0.5, and 2.2 ± 0.4 versus 0 ng/mL ($P < .01$, MTT *v* OGTT), 15 and 30 minutes following the MTT versus OGTT (Fig 4). It is noteworthy that the saline control challenge induced only minimal change from baseline in comparison to the other treatments (1.3 ± 0.5 ng/mL, n = 8, 15 minutes after the challenge, NS). Interestingly, the MTT induced an elevation in plasma insulin levels, which was higher and maintained for a longer period of time (2.2 ± 0.4 ng/mL) compared to the OGTT (0 ng/mL, 30

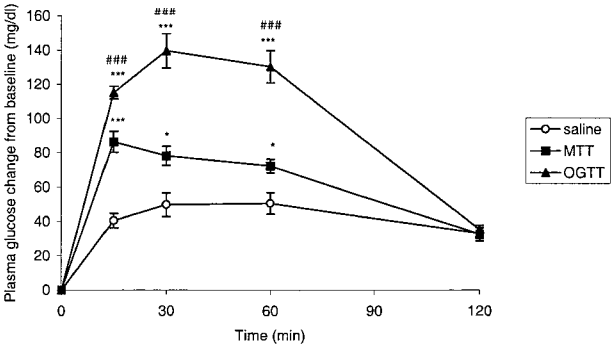

Fig 2. Change from baseline in plasma glucose levels *v* time following a MTT, an OGTT, or a saline control challenge. Results are the mean ± SE of 7-8 experiments. *$P < .05$ compared to saline, ***$P < .001$ compared to saline, ###$P < .001$ for MTT *v* OGTT.

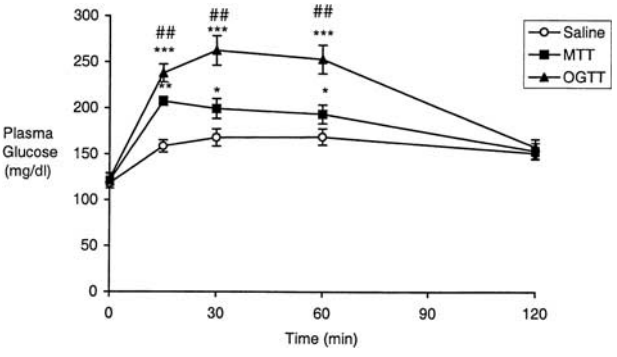

Fig 1. Plasma glucose levels before and following a MTT, an OGTT, or a saline control challenge. Results are the mean ± SE of 7-8 experiments. *$P < .05$ compared to saline, **$P < .01$ compared to saline, ***$P < .001$ compared to saline, ##$P < .01$ for MTT *v* OGTT.

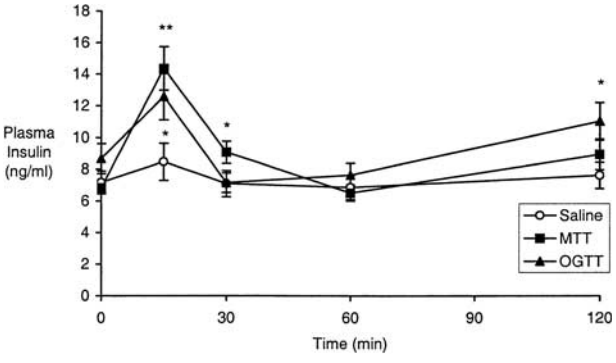

Fig 3. Plasma insulin levels before and following a MTT, an OGTT, or a saline control challenge. Results are the mean ± SE of 7-8 experiments. *$P < .05$ compared to saline, **$P < .01$ compared to saline.

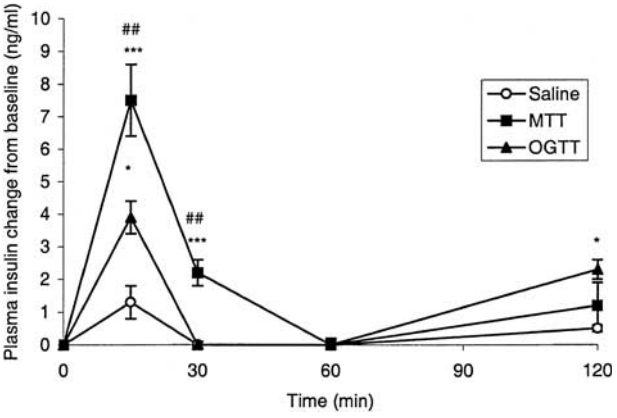

Fig 4. Change from baseline in plasma insulin levels *v* time following a MTT, an OGTT, or a saline control challenge. Results are the mean ± SE of 7-8 experiments. **P* < .05 compared to saline, ****P* < .001 compared to saline, ##*P* < .01 for MTT *v* OGTT.

minutes following the challenges, n= 7 or 8, $P < .01$, MTT *v* OGTT). In addition, plasma insulin levels returned to baseline at 60 minutes for both the MTT and the OGTT groups, and increased at 120 minutes (changes from baseline, 1.2 ± 0.7 *v* 2.3 ± 0.3 ng/mL, n = 7 or 8, MTT *v* OGTT, NS).

Finally, Fig 5 shows incremental AUC for glucose and insulin responses following the different challenges. The glucose AUC change from baseline was 1.6-fold greater in the OGTT group compared to the MTT group (Fig 5A) and the insulin AUC change from baseline following the MTT was 2-fold greater compared to that following the OGTT (Fig 5B).

DISCUSSION

In this study, the effects of an OGTT and a MTT were compared for glucose and insulin responses in one of the most often used models of insulin resistance, the Zucker fatty rat. An exaggerated insulin increase was observed for the MTT in comparison to the OGTT in the present study. In addition, a significant rise in glucose accompanies the exaggerated insulin response observed with the MTT.

It has been reported that, in the Zucker fatty rat (in comparison to lean controls), an amplified glucose intolerance and an increased hyperinsulinemia in response to an OGTT is observed as early as 7 to 8 weeks and more pronounced from 9 weeks on, reaching a near maximum at 12 to 13 weeks of age.[7] Interestingly, we observed similar results in response to a MTT in the same animal model (Berthiaume N, et al, unpublished observations, September 2000). For comparison and consistency with the literature, we chose to study this animal model at 8 to 9 weeks of age because it is a common age reported in the literature for the study of glucose and insulin responses to glucose challenges.[7,14,15]

An amplified increase in insulin was observed in conjunction with a reduced rise in glucose with a MTT in comparison to a typical glucose challenge. This indicates that glucose alone is more effective than a mixed liquid meal in driving the glucose response. A mixed liquid meal, therefore, is a more potent stimulator of insulin increase than glucose alone, while still significantly increasing glucose levels, though less than the OGTT. In fact, insulin levels induced by the MTT were elevated twice those induced by the OGTT. This 2-fold increase in plasma insulin levels by the MTT is similar or even slightly higher than that observed by other investigators following an OGTT of comparable CHO load in the Zucker fatty rat.[7,14,15] The important increase in insulin observed with the MTT can be attributed to the fat contained in a mixed liquid meal, while the reduced glucose response can be attributed to the well-reported slowed gastric emptying with a mixed nutrient challenge, in comparison to glucose alone, in humans as well as in rats.[8-10] The delay in gastric emptying of a liquid containing fat and protein is due in part to a redistribution of distal stomach contents back to the proximal stomach.[8] Human studies also suggest that increasing viscosity (mixed liquid meal *v* glucose solution) slows gastric emptying.[17,18]

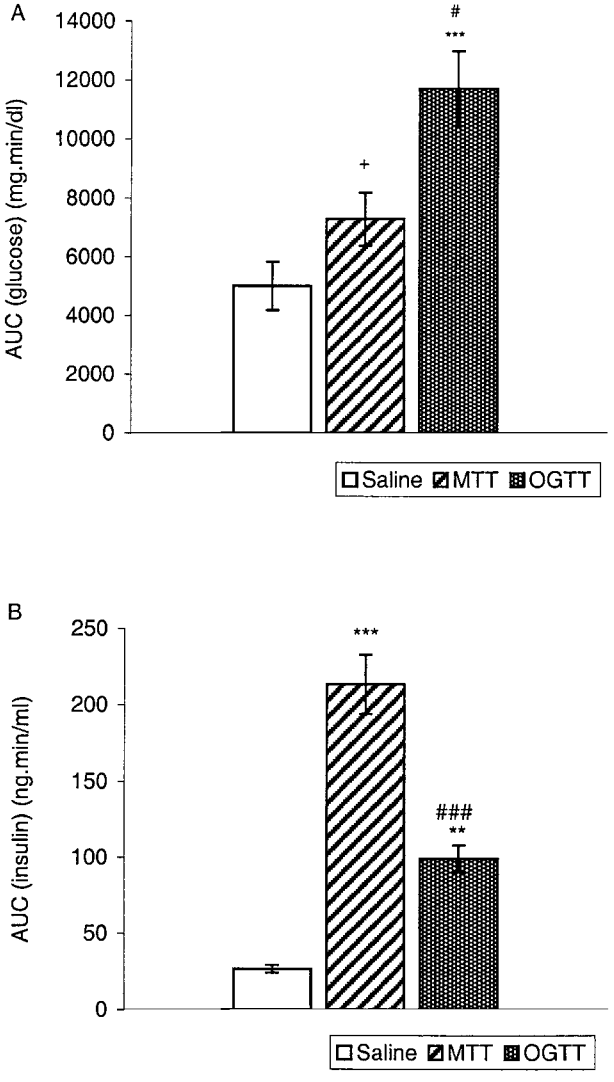

Fig 5. Incremental AUC for (A) glucose or (B) insulin induced by either a MTT, an OGTT, or a saline control challenge. Results are the mean ± SE of 7-8 experiments. ***P* < .01 compared to saline, ****P* < .001 compared to saline, #*P* < .05 for MTT *v* OGTT, ###*P* < .001 for MTT *v* OGTT, +*P* = .0835 for saline *v* MTT.

The insulin levels returned to baseline after 60 minutes following either the OGTT or the MTT, and increased slightly but not significantly over their respective baselines after 120 minutes following either the OGTT or the MTT. The physiological meaningfulness of the small significant difference between the insulin levels at 120 minutes between the OGTT and the saline control challenge is not clear.

The results of the current study in Zucker fatty rats are consistent with other OGTT results reported in rats as well as humans. Torgan et al[14] and Ivy et al[15] showed very similar glucose and insulin responses following a comparable glucose load in Zucker fatty rats of a similar age. Finally, Nolan et al showed a greater glucose response following an OGTT in comparison to a MTT (containing 33% of the caloric intake for a 24-hour period) in obese insulin-resistant humans.[19] The same group showed elevated insulin levels for a longer period of time following the MTT compared to the OGTT, as observed in the present study.[18] However, this previous study did not match volume or CHO load per kilogram of body weight and the macronutrient load of the liquid meal was not defined.

In conclusion, this is the first report, to our knowledge, comparing the effects of meal and glucose challenges, with matched CHO loads and routes of administration, on glucose and insulin responses in one of the most commonly used models of insulin resistance, the Zucker fatty rat. A mixed liquid meal, such as the MTT used in the current study, provides an effective complete nutrient challenge. A complete nutrient load including CHO, fat, and protein will have the advantage, over glucose alone, in inducing both glucose and insulin responses and thereby widening the window to detect differences in these parameters when investigating exaggerated insulin responses associated with the insulin-resistant and glucose-intolerant states like those found in the Zucker fatty rat. A MTT provides a more complete physiologic challenge (balanced macronutrient load) over that of an OGTT and a better capacity to detect differences in a model of insulin resistance such as the Zucker fatty rat. A MTT might be considered as an additional tool for the assessment of metabolic abnormalities in glucose-intolerant and insulin-resistant states such as those observed in the Zucker fatty rat.

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