Heritability of exercise training-induced adaptive change in glucose tolerance

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Heritability of Adaptive Change in Glucose Tolerance with Exercise Training

Submitted by

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HERITABILITY OF EXERCISE TRAINING-INDUCED ADAPTIVE CHANGE IN GLUCOSE TOLERANCE.

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Abstract

The magnitude of improvement in exercise training-induced glucose tolerance is widely variable among normal and diabetic individuals. The purpose of this study was to determine how much genetic factors influence improvement in glucose tolerance after exercise training. Seventy-nine offspring from 6 families of N/NIH rats underwent intraperitoneal glucose tolerance tests before and after 8 days of forced high-intensity intermittent swim training. Whole blood glucose was measured with an automatic glucometer and plasma insulin with a radioimmunoassay technique from blood samples obtained before and at 30 and 60 minutes after glucose injection (2g/kg body weight). Improvement in glucose tolerance was calculated from the difference between the post-training area under the curve (AUC) and the pre-training AUC. Narrow sense heritability, an index of genetic effects, estimated from regression of offspring on mid-parent values for training-induced adaptive change in glucose tolerance was 25% (r=0.90, p<0.05). A significant divergent response to selection was observed in the high versus low lines. There was a significant decrease in insulin response after exercise training and a significant increase in insulin sensitivity index after training, but no rank and training interaction was observed. The insulinogenic index, an index of insulin secretion capacity, showed no significant change after training in the high line, but a significant decrease was observed in the low line. There was a significant correlation between pre-training glucose tolerance and post-training glucose tolerance in the high line but not in the low line. The results demonstrate that genetic factors influence training-induced adaptive change in glucose tolerance to a moderate extent, and insulinogenic index and intrinsic glucose tolerance may be responsible for the differences between the high and low lines.
Introduction

Type 2 diabetes mellitus currently affects 17 million Americans. The disease is characterized by insulin resistance, which leads to impaired glucose tolerance. Type 2 diabetes mellitus occurs more in some families than others, which indicates that the disease may be heritable. It is currently unclear which aspects of glucose metabolism are responsible for the heritability of the disease. Researchers have investigated the genetic influences on fasting glucose and glucose tolerance. Snieder et al found 20-25% heritability in fasting insulin and 50% in fasting glucose (24). Hong et al found 25% heritability of glucose tolerance among whites and 48% among blacks of the HERITAGE Family Study (11). These studies indicate a moderate amount of heritability of glucose tolerance in the fasting state. However, glucose tolerance is a trait that adapts to environmental conditions such as diet and exercise. The magnitude of these adaptive changes may also be influenced by genetic factors.

Many studies have shown that glucose tolerance and insulin sensitivity improve with exercise training (1, 2, 6). Training may influence glucose metabolism by increasing the number of glucose transporter-4 (GLUT-4) proteins in skeletal muscle (5, 12), increasing the activity of insulin via more efficient signaling cascades (5, 17, 29), increasing insulin-independent signaling cascades (3, 9), and increasing the expression of genes which regulate glucose uptake and metabolism (10, 19, 28). There is wide variability among subjects in the magnitude of improvement in glucose tolerance after exercise training (1, 26). Tremblay et al found wide variability in adaptive change in glucose tolerance with exercise training among monozygotic twin pairs, though intrapair
comparisons revealed significant similarity in insulin sensitivity, which indicates a
genetic influence on the adaptive change (26).

Other investigators have researched the influence of specific genes on the
adaptive change in glucose tolerance such as peroxisome proliferator-activated receptor-γ
(14), β-3-adrenergic receptor (15, 27), angiotensin-converting enzyme genotypes (7, 13,
20, 22) and uncoupling protein-1 (15). Studies exploring the effects of specific genes do
not explain the all of the adaptive changes with exercise training in biological variables of
glucose metabolism, such as increased insulin sensitivity, decreased insulin response and
glucose tolerance. Further studies are needed to determine the heritability of the complex
trait of adaptive change in glucose metabolism with exercise training.

The purpose of this study was to determine the heritability of improvement in

Materials and Methods

Animals Seventy-nine 10-12-week-old rats from 6 families were bred in the Medical
College of Ohio animal facility from parents of the N/NIH strain with known training-
induced change in glucose tolerance. The rats were housed at a constant 22°C with a
fixed 12 hour on, 12 hour off light/dark cycle and had free access to food and water.

Intraperitoneal glucose tolerance test (IPGTT) An IPGTT was performed after overnight
fast of 16-18 hours (16-18 hours after the last exercise session) before and after exercise
training. Rats were anaesthetized by intraperitoneal injection of pentobarbital (20-30
mg/kg BW) and placed in a Plexiglas rat holder. The tail was warmed in a water bath
maintained at 40°C for 5 minutes and a catheter was inserted into a tail vein for blood collection. Blood was collected before and at 30 and 60 minutes after glucose was injected intraperitoneally (2g/kg BW). Whole blood glucose concentration was immediately measured using an Accu-Chek Advantage automatic glucometer (Roche Diagnostics Corporation, Indianapolis, IN).

**Insulin Assay** Blood samples were centrifuged and plasma fractions were collected and stored at –80°C for later analysis with a rat insulin radioimmunoassay kit (Linco Research Inc., St. Charles, MO).

**Swim training** The swim training protocol was adapted from Kawanaka et al (16). Rats swam 15 minutes in a barrel filled with ~45cm of water at 35°C in order to acclimatize them to swimming before training. Rats swam 8 bouts on days 1 and 2 and 10 bouts on days 3-8 of 20 seconds with 20-second rest periods between bouts while bearing weight equal to 14% of body weight at the base of the tail.

**Data analysis** The area under the glucose tolerance curve (AUC) was calculated using the trapezoid rule and a change in glucose tolerance was obtained from the difference between post-training AUC and pre-training AUC (post AUC-pre AUC) (25). The slope of a linear regression line between the mean of changes in glucose tolerance in the progeny of each family and a mid-parent value was used to determine narrow sense heritability of training-induced adaptive change in glucose tolerance (8). Data were further analyzed using 6 (family or rank) x 2 (training) ANOVA to determine the main effect of training and an interaction between rank (family) and training. This was followed by a paired t-test if a significant difference was found. Changes in insulin after training were assessed in the same way as changes in glucose tolerance. Insulin
sensitivity index (ISI) was determined using the formula $\text{ISI} = \frac{10,000}{(\text{fasting glucose} \times \text{fasting insulin})^{1/2}}$ from Matsuda (18). Insulinogenic index (IGI) was calculated from the widely-used formula $\left(\frac{[\text{insulin}_{30\text{min}}] - [\text{insulin}_{0\text{min}}]}{([\text{glucose}_{30\text{min}}] - [\text{glucose}_{0\text{min}}])}\right)$ (23). All statistical significant differences were determined at an $\alpha$ level of $p \leq 0.05$ unless otherwise noted. Data were presented in mean±SE unless otherwise noted.

**Results**

Reliability Five rats were used to test the reliability of the IPGTT. The mean AUC of trial one was $13218.0 \pm 2010.4 \text{ mg·dl/min}$ (mean±SD) and the mean AUC of trial 2 was $12441.0 \pm 2041.4 \text{ mg·dl/min}$ (mean±SD). The coefficient of variation for trial 1 was 15.2% and for trial 2 16.4%. There was no statistically significant difference in glucose AUC between the two trials.

Glucose tolerance Figure 1 displays the mean glucose tolerance curves for the offspring of each family, ranked according to the parent’s response to training. Rank 1 had significant improvement in whole blood glucose concentration at all time points of IPGTT. Rank 2 had significant improvement at 0 and 60 minutes. Rank 3 had significant improvement at 60 minutes. Rank 4 showed significant improvement at 60 minutes. Ranks 5 and 6 showed no significant improvement at any time point of the IPGTT.

Area under the glucose tolerance curve Figure 2 shows the change in glucose AUC after training. The improvement (post AUC-pre AUC) is significant for ranks 1 ($-2529 \text{ mg·dl/min} \pm 630, p<0.01$), 2 ($-1397 \text{ mg·dl/min} \pm 584, p<0.05$) and 3 ($-2234 \text{ mg·dl/min} \pm$
809, p<0.05), while ranks 4, 5 and 6 showed no significant improvement. Significant rank by training interaction occurred (p<0.05), indicating that each rank showed a different magnitude of change in glucose tolerance in response to training.

**Insulin Response** The insulin response to training was calculated using the difference between insulin AUC after training and the insulin AUC before training (post AUC-pre AUC). Figure 3 displays the overall improvement in plasma insulin concentration for each rank. All individuals as a group had significantly lower plasma insulin concentrations after training (p<0.01), but no interaction between rank and family existed.

**Heritability of adaptive change in glucose tolerance** Figure 4 displays a plot of improvement in glucose tolerance (post AUC-pre AUC) of offspring versus the mean of the improvement of parents (mid-parent value). A linear regression equation between the mean of the offspring and the mean of the parents was: offspring improvement = [0.25 x mid-parent value] –421, r=0.90, p<0.05. The slope of the linear regression represents heritability, indicating that 25% of the variability of the phenotype in the offspring is due to genetic factors.

**Divergent response to selection** Figure 5 displays the divergent response to selective breeding. The mean change of the offspring of the high line (ranks 1, 2 and 3) and low line (ranks 4, 5 and 6) were significantly different (-1832.63 mg·min/dl ± 408.73, n=40 versus –566.15 mg·min/dl ± 433.93, n=39, p<0.05).

**Insulin sensitivity index** Figure 6 displays the improvement in insulin sensitivity after training. Significant improvement was achieved among all individuals as a group (p<0.05). Each rank showed a tendency to increase, but no statistically significant
improvement was found, with the exception of rank 5. No interaction was observed between rank and training. The high line improved 30%, from 1.97 mg·min/dl ± 0.20 to 2.56 mg·min/dl ± 0.22 (p<0.05), and the low line improved 26%, from 1.97 mg·min/dl ± 0.19 to 2.49 mg·min/dl ± 0.20 (p<0.01). There was no statistically significant difference between the high and low lines.

**Insulin Secretion Capacity** Insulin secretion capacity is reflected in the value of the insulinogenic index, which is calculated by the change in insulin over the change in glucose during the first 30 minutes of the glucose tolerance test. There was no significant change in insulinogenic index in any rank except rank 5. The low line decreased 18%, from 0.92±0.10 pre-training to 0.75±0.08 post-training (p<0.05). There was no significant change in IGI in the high line.

**Intrinsic Glucose Tolerance** The relationship between pre-training glucose tolerance and post-training glucose tolerance is shown in Figure 7 (high line) and Figure 8 (low line). A significant relationship was observed between pre-training glucose tolerance and post-training glucose tolerance in the high line (r=0.51, p<0.001), while there was no significant relationship observed for the low line(r=0.26, p>0.05). These data suggest that the magnitude of change in glucose tolerance is related to the pre-training glucose tolerance.

**Discussion**

Wide variability exists among individuals in adaptive change in glucose tolerance after exercise training. This variability is due to both environmental factors, such as training status of the individual, nutritional status, variations in training protocols, muscle composition, etc. and genetic factors. The purpose of this study was to determine how
much genetic factors account for the magnitude of training-induced adaptive change in glucose tolerance by estimating the heritability of improvement in glucose tolerance after exercise training.

There was a high significant correlation of exercise training-induced change in glucose tolerance between parents and offspring ($r=0.90, p<0.05$), as displayed in Figure 4. The slope of the regression line shows the magnitude of heritability, which was 25%. The heritability found in this study is similar to heritability estimates reported by Snieder (20-50%) and Hong (25-48%) for basal glucose tolerance (11, 24). Tremblay et al found that improvement in glucose and insulin with exercise training was more similar within twin pairs than among pairs, which supports the implication that adaptive change in glucose tolerance after exercise training is a heritable trait (26). To further understand which factors are involved in different magnitudes of adaptive change in glucose tolerance for each rank after training, several variables associated with glucose metabolism were analyzed.

Adaptive change in glucose tolerance with exercise training is influenced by the sensitivity of peripheral tissues to insulin, the sensitivity of the pancreas to changes in blood glucose concentration, and intrinsic glucose tolerance. All individuals as a group had significantly decreased insulin concentration in response to a glucose challenge after exercise training. This is in accordance with other studies, which show an adaptive decrease in insulin secretion with exercise training (1, 2, 6, 15, 21, 26). Decreased insulin production with an equivalent glucose challenge would suggest increased sensitivity of peripheral tissues to insulin. The insulin sensitivity index (ISI), an index of sensitivity of peripheral tissues to insulin, significantly improved for all individuals as a group. ISI was
not significantly different between high and low lines, suggesting that insulin sensitivity
does not explain the differences in adaptive changes in glucose tolerance in the high and
low lines. The insulinogenic index (IGI) reflects pancreatic sensitivity to a glucose
challenge in terms of insulin secretion. No significant change in IGI is noted for the high
line. However, a significant change is observed for the low line (18% decrease from 0.92
± 0.10 to 0.75 ± 0.08, p<0.05). This suggests that among the individuals in the low line,
the pancreas secreted relatively less insulin in response to the change in glucose when
compared to the high line. This might be one of the factors that explains the different
magnitudes of change in glucose tolerance between the two lines. The final factor
examined was intrinsic glucose tolerance (Figures 7 and 8). Among the individuals of the
high line, the plot of pre-training glucose tolerance to post-training glucose tolerance
revealed a slope less than 1. This indicates that a significant relationship exists between
post-training glucose tolerance and intrinsic glucose tolerance (r=0.51, p<0.001). If
intrinsic glucose tolerance did not influence the adaptive change in glucose tolerance
after training, a slope of 1 would have been observed. Among the individuals of the low
line, no significant relationship was observed between pre-training glucose tolerance and
post-training glucose tolerance(r=0.26, p>0.05). The difference between high and low
lines with respect to intrinsic glucose tolerance may also partially explain the divergence
observed between the two lines.

The significant divergence of the high and low lines of animals in response to
selective breeding indicates that N/NIH rats may be good substrates for creation of an
animal model used to determine the genes responsible for training-induced changes in
glucose tolerance, according to the description of the creation of animal models for
complex, quantitative physical traits by Britton and Koch (4). The use of animal models allows selective breeding for a specific trait. Inbred strains with little genetic and phenotypic variation in the trait of interest can be created after 20 generations of selective breeding. The individual strains may then be genetically compared to determine the loci of the genes that account for the differences.

In conclusion, improvement in glucose tolerance with exercise training is a trait with moderate heritability. Some of the intermediate phenotypes that may explain the variability in the trait are pancreatic sensitivity to glucose and intrinsic glucose tolerance. Further studies using animal models are needed to determine the genes responsible for adaptive change in glucose tolerance after training.

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References


**Figure Legend**

**Figure 1** Figure 1 represents the mean glucose tolerance curve before and after training for the offspring of each rank. One asterisk (*) indicates significance at p<0.05. Two asterisks (**) indicate significance at p<0.01. Rank 1 showed significant improvement at all three time points of IPGTT after training. Ranks 2 and 3 showed significant improvement at 0 and 60 minutes, while rank 4 showed significant improvement only at 60 minutes. Ranks 5 and 6 had no significant change with training.

**Figure 2** Figure 2 represents the change in glucose tolerance after training for each rank. One asterisk (*) indicates significance at p<0.05. Two asterisks (**) indicate significance at p<0.01. Ranks 1, 2 and 3 showed significant improvement in glucose tolerance after training. The families had a different response to training, as evidenced by the different magnitudes of change, which indicates that training and rank have interacted.

**Figure 3** Figure 3 displays the change in insulin concentration after training, or the insulin response to training. While a significant improvement among all individuals as a group was observed (p<0.01), the change was not significant for any rank except rank 5. No training and rank interaction was observed, as indicated by the similar amount of change among the ranks.

**Figure 4** Figure 4 represents the heritability of the trait of adaptive change in glucose tolerance with exercise training. The black dots are the values of the change in glucose tolerance for individual offspring (y-axis), plotted against the mean change for the parents (x-axis). The open circles represent the mean change in glucose tolerance for the offspring of each rank. The linear regression obtained from this plot represents the heritability of the trait, which was found to be 25% (r=0.90, p<0.05)
Figure 5 Figure 5 represents divergent glucose tolerance responses to training between the high line (ranks 1, 2, and 3) and the low line (ranks 4, 5, and 6). The difference in response between the two lines was significant (p<0.05).

Figure 6 Figure 6 represents the change in insulin sensitivity index for each family after training. The change was significant for all individuals as a group, but not significant for any rank except rank 5. There was no interaction between rank and training, as indicated by the similar change among all ranks.

Figure 7 Figure 7 represents the relationship between pre- and post-training glucose tolerance for the high line. A significant relationship was observed for pre- and post-training (r=0.51, p<0.001), indicating that the intrinsic glucose tolerance of individuals of the high line influenced the glucose tolerance response to training.

Figure 8 Figure 8 represents the relationship between pre- and post-training glucose tolerance for the low line. No significant relationship was observed between pre- and post-training glucose tolerance, indicating that the intrinsic glucose tolerance of individuals of the low line did not influence the glucose tolerance response to training.
Fig. 1. Glucose tolerance curve before and after training in the offspring
Fig. 2. Glucose area under curve before and after training
Figure 3: Insulin response before and after training
Offspring improvement = (0.25 x mid-parent value) - 421

r = 0.90; P<0.05; Heritability = 25%

Figure 4: Heritability of training-induced change in glucose tolerance
Figure 5: Divergent response to selection

Training-induced improvement, (mg/dl).min
Figure 6: Insulin Sensitivity Index before and after training
Figure 7: Intrinsic glucose tolerance of the high line
Figure 8: Intrinsic glucose tolerance of the low line