Effect of exercise training on metabolic intermediate phenotypes in inbred rat strains

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FINAL APPROVAL OF THESIS
Master of Science in Biomedical Sciences

Effect of Exercise Training on Metabolic Intermediate Phenotypes in Inbred Rat Strains

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In partial fulfillment of the requirements for the degree of Master of Science in Biomedical Sciences

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Effect of Exercise Training on Metabolic Intermediate Phenotypes in Inbred Rat Strains

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University of Toledo, Health Science Campus

2007
DEDICATION

I dedicate this thesis to my parents, Dipu and Dana Ghosh, for their undying love and support.
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Introduction

Approximately 21 million people are afflicted with diabetes mellitus in the US today. This exceedingly high number, roughly 7% of the American population, continues to rise. There are two kinds of diabetes: Type 1 diabetes results from an insulin deficiency due to autoimmune destruction of pancreatic beta cells, responsible for insulin secretion; Type 2 diabetes mellitus (T2DM) results from failure of normal glucose homeostasis, insulin secretion and insulin action. Consequences of such impaired actions result in the gradual onset of hyperglycemia and hyperinsulinemia (Tennyson 2002). T2DM is the outcome of decreased insulin sensitivity mainly in skeletal muscle and accounts for the majority of individuals with diabetes mellitus. Resulting symptoms may include polyphagia, polyuria, polydipsia, fatigue, blurred vision, and weight loss. Abnormalities arising from insulin resistant states and T2DM are commonly treated by implementing therapies including diet restriction, physical activity, and drug regimens (Kendall and Bergenstal 2001).

Insulin action:

Insulin is secreted by pancreatic beta cells in response to elevated blood glucose levels following a meal. Insulin controls glucose homeostasis by decreasing hepatic glucose output and increasing the rate of glucose uptake by skeletal muscle and adipose tissues from the blood. Glucose uptake is dependent on insulin mediated Glut-4 translocation to the surface of muscle and adipose cells (Pessin and Saltiel 2000). Insulin also plays a major role in lipid homeostasis in the body, stimulating an increase in lipid synthesis in both liver and adipose cells and decelerating free fatty acid release from triglycerides in these tissues (Pessin and Saltiel 2000).
Insulin action begins with its binding to the insulin receptor on the cell surface. Insulin binds to the extracellular alpha subunits of the insulin receptor activating the intracellular tyrosine kinase element of the beta subunits. The insulin receptor phosphorylates several primary substrates on tyrosine such as members of the insulin receptor substrate family (IRS1/2/3/4) and Shc protein isoforms, resulting in the initiation of a series of signaling pathways (Pessin and Saltiel 2000; Cefalu 2001).

The major tissue responsible for insulin mediated glucose uptake is skeletal muscle (Kennedy, Hirshman et al. 1999; Terada, Yokozeki et al. 2001; Henriksen 2002). Glucose uptake into cells through the plasma membrane occurs through protein transport molecules, specifically SLC2A1, SLC2A4, SLC2A5 also known as Glut-1, Glut-4 and Glut-5, respectively (Barnard and Youngren 1992). Glut-1 and Glut-5 transporters are located in the cell membrane and are typically responsible for glucose uptake under basal conditions (Barnard and Youngren 1992; Hayashi, Wojtaszewski et al. 1997; Kennedy, Hirshman et al. 1999). Glut-4 transporter, however, is located intracellularly. In response to insulin or exercise stimulation, Glut-4 is translocated to the plasma membrane (Kennedy, Hirshman et al. 1999; Thorell, Hirshman et al. 1999; Cefalu 2001). Proximal steps in the insulin signaling pathway for glucose transport include phosphorylation of several substrates on tyrosine residue including insulin receptor substrate -1(IRS-1) associated with phosphatidylinositol 3’- kinase (PI 3-kinase) (Thorell, Hirshman et al. 1999). Phosphorylation of IRS-1 activates PI 3-kinase (Hayashi, Wojtaszewski et al. 1997; Cefalu 2001; Henriksen 2002), which, in turn, activates Akt, a downstream effector of PI 3-kinase, through interaction with other signaling proteins. Finally Akt substrate 160 (AS160), becomes phosphorylated by Akt, causing Glut-4 translocation to the

**Insulin Resistance:**

Insulin resistance is defined as a state in which greater than normal levels of insulin are required to elicit a normal response (Peters 2000). Insulin resistance is associated with a number of metabolic disorders including glucose intolerance, hypertension, visceral obesity, T2DM, hyperinsulinemia and dyslipidemia (Lebovitz 1999; Henriksen 2002; Tennyson 2002). Close association between insulin resistance and T2DM often implicate it as a precursor to the development of T2DM (Lebovitz 1999; Henriksen 2002). Impaired insulin action in peripheral tissues such as skeletal muscles and other organs such as the liver and adipose tissues has been considered to be a major factors in the progression of insulin resistance (Cefalu 2001).

According to the insulin signaling pathway as explained above, there are a number of possible steps that may be associated with insulin resistance, ranging from decreased insulin binding to its receptors to abnormality in phosphorylation status of AS160, the most distant downstream effector of insulin receptor. Most often insulin resistance commonly found in association with obesity is attributed to defects in insulin signaling proteins downstream to insulin receptors such as IRS-1 and IRS-1-associated PI 3-kinase (Paz, Hemi et al. 1997; Cusi, Maezono et al. 2000; Qiao, Zhande et al. 2002).

Pathophysiology associated with insulin resistance likely results from both genetic predisposition and environmental factors including high fat diet and physical inactivity (Kendall and Harmel 2002). Both variables, degree of genetic risk and environmental factors, may lead to a decreased tissue response to an insulin stimulus and
a decline in glucose-induced insulin secretion resulting in the development of insulin resistance and T2DM (LaMonte, Blair et al. 2005). Decreased tissue response to insulin indicates diminished cellular glucose uptake and decreased cellular glucose transport resulting in eventual hyperglycemia, characteristic in the insulin resistant state. Prolonged hyperglycemia may lead to impairment in beta cell function and consequential insulin deficiency (LaMonte, Blair et al. 2005). Physical inactivity and insulin resistance have also been associated with decreased activity in muscle oxidative enzymes in the mitochondria (Short, Vittone et al. 2003). This series of events aids in the progression of pathology associated with T2DM, obesity and insulin resistance.

**Treatment Options for Insulin Resistance/T2DM:**

Treatment options for insulin resistance and T2DM are primarily geared to alleviate symptoms of glucose intolerance, hyperglycemia, dyslipidemia and hypertension. Therapies include diet restrictions and increased physical activity to induce moderate body weight loss that could result in increased insulin sensitivity and improved glucose tolerance (Kendall and Bergenstal 2001).

A variety of drug therapies targeting different regulatory pathways involved in glucose homeostasis can also be implemented to target specific metabolic complications. Management and treatment of T2DM can be quite complicated due to the range of metabolic pathways that T2DM can affect, leading to: hyperglycemia, dyslipidemia, hypertension and microvascular problems (Kendall and Bergenstal 2001). Drug therapy options to alleviate hyperglycemia and insulin resistance range from monotherapy to combinations of different oral antihyperglycemic agents (Lebovitz 1999). Available drug therapy achieves glycemic targets by regulating mechanisms to increase insulin secretion.
and decrease insulin resistance. Drug treatments are administered on a case-by-case basis in combination with lifestyle intervention geared to reverse effects of hyperglycemia, dyslipidemia and hypertension (Lebovitz 1999; Kendall and Bergenstal 2001).

**Adaptation to Exercise:**

A number of possible biological mechanisms may contribute to increase in insulin sensitivity and improvement in glucose homeostasis in response to exercise training (LaMonte, Blair et al. 2005). Both structural and biochemical changes in skeletal muscle can result from exercise training. Structural changes in skeletal muscle involve an increase in fiber size, fiber transformation, an increase in capillary density with a subsequent increase in blood flow (Henriksen 2002; LaMonte, Blair et al. 2005). Biochemical changes such as increase in insulin signaling kinetics, increase in non-insulin signaling kinetics and increase in the activities of enzymes associated with glucose metabolism, are a few examples of cellular changes that may occur as a result of exercise training. Other benefits induced by exercise training include an increase in maximal oxygen uptake, hepatic adaptation such as a decrease in hepatic secretion of glucose, and improvement in the conditions of hyperglycemia, visceral obesity, dyslipidemia and hypertension (LaMonte, Blair et al. 2005). Specific cellular mechanisms responsible for improvement observed in glucose tolerance and insulin sensitivity in response to exercise training are elusive. Furthermore, it has been shown that an interaction between exercise and genetic factors plays an important role in improvement in insulin action and glucose metabolism in response to exercise training (Walston, Silver et al. 2000; Dengel, Brown et al. 2002; Kahara, Takamura et al. 2003; Lakka, Rankinen et al. 2003). Currently, a genetic model, which may facilitate the
identification of genotypic determinants for exercise training-induced improvement in insulin action and glucose tolerance, is not available. The present study was designed to test the feasibility of creation of an animal genetic model by testing the inbred strains of rats and to characterize metabolic intermediate phenotypes that may explain difference in exercise training-induced change in glucose tolerance between two inbred strains of rats.
Purpose of Study:

The purpose of this study was to determine whether training induced adaptive change in glucose tolerance was due in part to genetic factors. If the preceding were true, we also aimed to determine which intermediate phenotypes accounted for this difference in adaptation.

We accomplished this by investigating two different strains of rats, Copenhagen (COP) and DA. Aerobic running capacity has been previously evaluated in both DA and COP rat strains, showing the DA rats possess a twofold greater intrinsic capacity for endurance treadmill running than the COP, providing genetic models representing both high and low aerobic capacities (Barbato, Koch et al. 1998; Koch, Green et al. 2005). Following establishment of a genetic role in training-induced adaptive changes in terms of glucose tolerance, potential intermediate phenotypes were examined in order to determine what is responsible for difference in glucose tolerance between the two strains. Intermediate phenotypes were chosen for evaluation based on their involvement in glucose metabolism.
LITERATURE REVIEW

DA and COP rats in relation to aerobic capacity:

Low aerobic capacity is associated with decreased cardiovascular fitness and metabolic function in humans (Barbato, Koch et al. 1998; Wisloff, Najjar et al. 2005). Aerobic capacity is a complex trait in that there are a number of genes responsible for variation in intrinsic aerobic capacity, separate from those genes that are responsible for adaptation of aerobic capacity in response to exercise training (Troxell, Britton et al. 2003). Gene interactions and environmental factors contribute to overall aerobic performance (Britton and Koch 2001; Ways, Cicila et al. 2002; Ways, Smith et al. 2007). Previous studies in both mice and humans conducted suggest genetic variance as being a major cause in variability in exercise performance between individuals (Bouchard, Lesage et al. 1986; Bouchard, An et al. 1999; Lightfoot, Turner et al. 2001).

Because aerobic capacity is a polygenic trait, variation resulting in distribution of this phenotype ranges from low to high (Barbato, Koch et al. 1998). Aerobic running capacities of eleven inbred rat strains (Barbato, Koch et al. 1998) were evaluated, and COP and DA strains were found at opposite ends of the spectrum; COP rats exhibited the lowest aerobic capacity and DA showed the highest. Chromosomal regions containing genes responsible for variation in aerobic capacity have been previously identified; on rat chromosomes 3 and 16 (Ways, Cicila et al. 2002; Ways, Smith et al. 2007). Though the rat and human genomes are not completely homologous, rat models provide a template from which information can be extrapolated and a general understanding can be gained and applied to human models (Jacob and Kwitek 2002).
It has been reported that low intrinsic aerobic capacity is associated with the development of the characteristics of metabolic syndrome in rats selectively bred for low aerobic capacity (Wisloff, Najjar et al. 2005). No previous study has been conducted on DA and COP with an aim of testing whether or not these two strains of rats can be employed as an animal genetic model, which aids the identifications of genotypic determinants for training-induced improvement in glucose tolerance and intermediate metabolic phenotypes that might constitute a difference between the these strains if present at all in response to exercise training.

**Interaction between exercise and genetic factors:**

A number of genetic factors associated with glucose metabolism and insulin sensitivity, in response to exercise training, have been previously identified. Angiotensin-Converting Enzyme (ACE) is known to influence blood pressure regulation, which is frequently associated with insulin sensitivity (Dengel, Brown et al. 2002). Differences in ACE levels, in response to exercise training, among individuals were found to be due, in part, to polymorphisms, specifically insertions/deletions, in intron 16 of the ACE gene (Dengel, Brown et al. 2002). Chromosomal region 7q21-q31 has also been found to be strongly linked with fasting insulin in response to exercise training in white people (Lakka, Rankinen et al. 2003). This region has been previously shown to contribute to a number of other metabolic phenotypes including leptin and blood pressure in Hispanic families. Chromosome 7q21-q31 also houses a number of other genes that influence glucose metabolism (Lakka, Rankinen et al. 2003). Peroxisome proliferators-activated receptor-\(\gamma\) (PPAR\(\gamma\)) is a known regulator of adipose cell differentiation and a Pro12Ala polymorphism present in this gene has been identified to influence insulin sensitivity and

Because training-induced improvement in glucose metabolism is a polygenic trait, it is expected that multiple genes influence observed adaptive changes in glucose metabolism and associated biological variables. To have a better approach, it is necessary to implement an animal genetic model which may facilitate the identification of potential genes responsible for various changes in response to exercise training.

**Insulin:**

Exercise training is known to result in an improvement of insulin sensitivity. A number of mechanisms attribute to such adaptation in the body. Generally, plasma insulin levels decrease during exercise, indicative of increased insulin sensitivity (Wojtaszewski, Nielsen et al. 2002). Insulin levels are determined by insulin secretion into the blood, body distribution and clearance (Castillo, Scheen et al. 1994). Insulin and C-peptide are secreted simultaneously by pancreatic beta cells in equimolar amounts as proinsulin. The enzymatic cleavage of proinsulin results in the formation of peptides, insulin and C-peptide (Palmer, Fleming et al. 2004). From the pancreas, these peptides pass through the liver, at which point, insulin is partially cleared and metabolized. C-peptide and remaining insulin peptides are then distributed to the body. *In vivo* insulin levels are commonly measured to evaluate beta cell function (Castillo, Scheen et al. 1994). Insulin plasma levels can be representative of insulin secretion from the beta cell. But, C-peptide levels reflect insulin secretion from the pancreas more accurately than insulin levels.
because C-peptide is not metabolized by the liver and is removed slowly from circulation. Insulin clearance rates, however, can be evaluated by examining the C-peptide/insulin molar ratio, as this ratio decreases with a decrease in insulin clearance and increases with an increase in insulin clearance (Castillo, Scheen et al. 1994).

Insulin clearance from circulation is mediated by carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1), a trans-membrane glycoprotein, located in the liver (Najjar 2002; Poy, Yang et al. 2002; Dai, Abou-Rjaily et al. 2004). Najjar and colleagues have shown that CEACAM1 plays a critical role in glucose homeostasis and insulin action (Najjar 2002; Poy, Yang et al. 2002; Dai, Abou-Rjaily et al. 2004; Park, Cho et al. 2006). An importance of the biological role of CEACAM1 for insulin regulation, and consequently, the regulation of glucose and fat metabolism has been demonstrated in CEACAM1-inactivated transgenic mice. These mice developed hyperglycemia in random fed state, visceral fat accumulation, and dyslipidemia along with hyperinsulinemia caused by the lack of insulin clearance due to the liver-specific overexpression of dominant negative phosphorylation-defective S503A-CEACAM1 mutant (Najjar 2002; Dai, Abou-Rjaily et al. 2004; Park, Cho et al. 2006). Furthermore, it has been found that the expression of CEACAM1 is reduced in rodents such as Zucker rats in which obesity-associated development of diabetes has been found (unpublished, Najjar). Effect of exercise training on the expression of CEACAM1 is currently unknown. Thus it was investigated to determine whether CEACAM1 expression would be modulated by exercise training and associated with training-induced improvement in glucose tolerance.
**Glucose Metabolism:** During exercise glucose and fat serve as fuel in working muscles. It has been shown that training causes adaptive changes in several steps in glucose and fat metabolic pathways (Figure 1). Glucose metabolism starts from the entry of glucose into muscle cells by glucose transporters and is converted by hexokinase into glucose-6-phosphate (G-6-P), which may either undergo glycolysis to generate ATP or be incorporated into glycogen by glycogen synthase (GS) (Kim, Youn et al. 2000).

**Figure 1: Metabolic Pathways**

A number of metabolic pathways are responsible for maintaining whole body homeostasis. Metabolism of both glucose and fats contribute to energy (ATP) production. Glucose is broken down by conversion into glucose-6-phosphate. This intermediate then either undergoes glycolysis to eventually generate ATP, or is converted into glycogen via glycogen synthase enzyme. Lipids are broken down into free fatty acids to endure β-oxidation producing acetyl-CoA. Based on energy requirements, acetyl-CoA either moves through the citric acid cycle to generate ATP or undergoes lipogenesis.
Glucose Transport:

As previously mentioned, the primary tissue responsible for insulin mediated glucose uptake is skeletal muscle (Kennedy, Hirshman et al. 1999; Terada, Yokozeki et al. 2001; Henriksen 2002). Exercise training has been shown to improve responsiveness to insulin by increasing insulin-mediated glucose uptake (Luciano, Carneiro et al. 2002). Though a number of different variables may be responsible for this cellular adaptation observed in the skeletal muscle tissue, it is likely that some of these adaptive changes result from increase in activity and expression of proteins involved in the insulin signaling pathway and the glucose transporter, SLC2A4, also known as Glut-4 (Luciano, Carneiro et al. 2002; Peres, de Moraes et al. 2005). Increased insulin binding to its receptor has been detected in response to training, leading to increased insulin receptor (IR) autophosphorylation and subsequent phosphorylation of insulin receptors (IRS-1 and IRS-2). Enhanced IRS-1/2 phosphorylation results in enhanced activation of the PI3-kinase and Akt, both of which have been implicated in Glut-4 translocation to the membrane for glucose uptake (Luciano, Carneiro et al. 2002)

Citrate synthase:

Citrate synthase (CS) is a major regulatory enzyme involved in glucose metabolism (Siu, Donley et al. 2003). CS initiates the reaction in which oxaloacetate and acetyl CoA are condensed to form citrate in the citric acid cycle (Siu, Donley et al. 2003). This enzyme’s activity and protein expression levels are commonly measured as a mitochondrial marker to determine muscle oxidative and respiratory capability. Frequently insulin resistance or diabetes is associated with reduced muscle oxidative capacity due to a decrease in the number of mitochondria and key oxidative enzymes
such as citrate (Simoneau and Kelley 1997). Endurance exercise training results in an elevation in both CS enzyme activity and protein expression, likely due to an increase in rate of protein synthesis, in skeletal muscles (Siu, Donley et al. 2003). Since glucose is oxidized through citric acid cycle, one can expect that training-induced enhanced muscle oxidative capacity will improve insulin sensitivity and glucose tolerance.

**Glycogen synthase:**

Insulin resistance can often be attributed to reduced glycogen synthesis, another element of the glucose metabolism pathway (Kida, Esposito-Del Puente et al. 1990; Thorburn, Gumbiner et al. 1991). Such results may be implicated by an impairment of insulin-induced stimulation of glycogen synthase (GS), the enzyme responsible for glycogen synthesis. GS activity is controlled both allosterically and covalently by glucose-6-phosphate and phosphorylation, respectively (Wojtaszewski, Nielsen et al. 2002). Insulin increases GS activity by inhibiting its phosphorylation (Wojtaszewski, Nielsen et al. 2002).

During exercise, glycogen stores serve as an energy source for contractile activity. Glycogen synthesis is in fact increased after exercise, under glycogen depleted conditions, compared to resting state (Wojtaszewski, Nielsen et al. 2002). Because low glycogen levels potentially hinder exercise performance, glycogen resynthesis is crucial. Glycogen resynthesis occurs most efficiently, however, after carbohydrate ingestion. With carbohydrate availability, glycogen resynthesis and GS enzyme efficiency is so great that often glycogen levels surpass those of the normal resting level (Wojtaszewski, Nielsen et al. 2002). This likely results from improved in insulin action due to training (Wojtaszewski, Nielsen et al. 2002). Exercise training has shown elevated levels of both
insulin stimulated glycogen synthesis and glycolysis, particularly via key enzymes such as glycogen synthase (Kraegen, Storlien et al. 1989; Kim, Youn et al. 2000).

**Lipid Metabolism:** Fat accumulation in visceral adipose tissues, the liver and skeletal muscles is associated with the development of insulin resistance, which, consequently, leads to the development of diabetes mellitus (Roden, Price et al. 1996). Thus, it is important to understand biological processes of the synthesis and degradation of fat in the body. Fat is synthesized primarily in the liver and adipose tissues and stored in the form of triglyceride, which can be used as energy source if demanded during exercise. Acetyl CoA carboxylase (ACC) is a key enzyme involved at an initial committed step in lipogenic pathway, which converts acetyl CoA to malonyl CoA (Lewis, Carpentier et al. 2002). ACC, which exists in two isoforms, ACCα, a predominant isoform in lipogenic tissues such as the liver, and ACCβ, a predominant isoform in skeletal muscles, is regulated by AMP-activated protein kinase (AMPK), which phosphorylates on serine residues of ACC resulting in an inactivation of the enzyme (Rasmussen and Winder 1997; Ruderman, Saha et al. 1999). Malonyl CoA, the product of ACC, is an inhibitor of carnitine-palmitoyl transferase-1 (CPT-1), which translocates long chain fatty acids into mitochondria for its oxidation (McGarry 1995; Rasmussen and Winder 1997; Ruderman, Saha et al. 1999).

The levels of blood lipids such as free fatty acids (FFA) and triglyceride (TG) in a fasted state reflect the *de novo* synthesis of fats by lipogenic enzymes in the liver and adipose tissues since blood FFA may result from either the diffusion of FFA present in the liver and adipose tissues into the circulatory system or the breakdown of blood TG by lipase (Wakil, Stoops et al. 1983; Hellerstein, Christiansen et al. 1991; Horowitz and
Plasma TG is regulated in a fasted state by the liver through *de novo* synthesis of very low density lipoprotein (VLDL), which contains the highest amount of TG among all lipoproteins (Hellerstein, Christiansen et al. 1991). Plasma FFA are typically cleared via esterification in the liver or oxidation by skeletal muscles (Lewis, Carpentier et al. 2002).

Skeletal muscle is not considered to be a major lipogenic tissue, but it plays an important role in the regulation of fat metabolism in the body. Skeletal muscle takes up circulating FFA as mentioned before, synthesizes fat and stores it as TG (Kennedy, Hirshman et al. 1999; Terada, Yokozeki et al. 2001; Henriksen 2002). During exercise, triglycerides are broken down into free fatty acids, which are metabolized through β-oxidation yielding acetyl-CoA, which, in turn, enters into the citric acid cycle for the production of ATP (Watt, Heigenhauser et al. 2002; Tamura, Tanaka et al. 2005). During low intensity exercise, muscle TG serves as predominant energy source for working muscles (Watt, Heigenhauser et al. 2002). This is achieved through the activation of AMPK due to a low intracellular energy level, i.e., an increase in AMP level and a decrease in ATP level, in working muscles (Musi, Hayashi et al. 2001; Durante, Mustard et al. 2002; Tomas, Tsao et al. 2002). The activated AMPK phosphorylates ACC, which, in turn, is inhibited, producing minimal amount of malonyl CoA. This relieves the inhibitory effect of malonyl CoA on CTP1, promoting the oxidation of FFA in mitochondria (McGarry 1995; Rasmussen and Winder 1997; Ruderman, Saha et al. 1999).

Exercise-induced activated AMPK affects not only fat metabolism but also glucose metabolism. It has been shown that exercise-induced improvement in insulin
sensitivity may be mediated, in part, by AMPK (Fisher, Gao et al. 2002). Exercise-induced activated AMPK contributes to increased glucose transport activating protein AS160, responsible for translocation of GLUT-4 to the plasma membrane, and fatty acid oxidation resulting in overall improved efficiency in glucose metabolism and tolerance (Tomas, Tsao et al. 2002; Bruss, Arias et al. 2005).

**Lipids and insulin resistance:**

It is well documented that the high levels of plasma FFA and TG have been linked to insulin resistance and impairment in glucose homeostasis (Kelley and Goodpaster 2001; Kelley, Williams et al. 2001; Straczkowski, Kowalska et al. 2001; Boden and Shulman 2002; McGarry 2002; Petersen and Shulman 2002; Tamura, Tanaka et al. 2005). However the mechanism underlying insulin resistance induced by increased plasma FFA or TG is unclear. This impairment may be attributed to interference with cellular glucose uptake since insulin-responsible tissues such as skeletal muscles and fat cells have limited capacity to transport and oxidize either plasma FFA or glucose (Lewis, Carpentier et al. 2002). It has been explained, according to Randle cycle, that excess FFA uptake by muscles causes an increase in the intermediate metabolites that exert an inhibitory effect on key enzymes in glucose metabolic pathway such as citrate, which inhibits the activity of phosphofructokinase (PFK), and acetyl CoA, which inhibits the activity of pyruvate dehydrogenase (Randle, Garland et al. 1963; Randle, Garland et al. 1965; Roden, Price et al. 1996). This explanation has been challenged by the results of recent studies of Shulman and colleagues, where the accumulation of free glucose and glucose-6-phosphate and the rate of glycogen synthesis have been measured under the conditions of high level of muscle FFA using $^{13}$C- and $^{31}$P-MRS techniques (Rothman,
Through these studies using humans and animals results have suggested that the accumulation of FFA and/or its metabolites activate serine-kinase such as protein kinase C-theta, which, in turn, inhibits the first step of glucose metabolism, that is, glucose transport into muscle cells (Rothman, Shulman et al. 1992; Rothman, Magnusson et al. 1995; Petersen, Hendler et al. 1998; Dresner, Laurent et al. 1999; Griffin, Marcucci et al. 1999). Apparently there is a paradoxical observation of the effect of muscle fat on glucose metabolism. In trained individuals the level of muscle TG is higher than that in untrained individuals without the development of muscle insulin resistance (Kelley and Goodpaster 2001). So others have postulated that an increased TG levels in skeletal muscle without high oxidative capacity may render a negative effect of on glucose metabolism and the combination of cyclic depletion and repletion of muscle TG occurring with regular exercise prevents the development of insulin resistance (Kelley and Goodpaster 2001).

In summary, exercise training can result in adaptive changes in a variety of mechanisms associated with glucose metabolism. After establishing a genetic element in exercise adaptation, it is necessary to investigate potential intermediate phenotypes responsible for observed adaptive changes represented in variance in glucose tolerance. Determining these specific mechanisms contributing to any difference seen in glucose tolerance adaptation will help provide a more concise model for body adaptation to exercise in terms of treatment for metabolic disorders and its relation to potential genetic variables.
MATERIALS

Insulin and C-peptide kits were purchased from Linco Research (St. Charles, MO). The Free Fatty Acid kits were purchased from Wako Chemicals USA, Inc (Richmond, VA). Chemicals in reagents used for citrate synthase, glycogen, ACC and triglyceride assays were purchased from Sigma (St. Louis, MO). Radioactive material $^{14}$C-NaHCO$_3$ used in the ACC assay was acquired from American Radiolabeled Chemicals Inc (St. Louis, MO). Western blotting reagents were purchased from BIORAD (Hercules, CA). Other general chemicals used in preparation of buffer solution were purchased from either Sigma (St. Louis, MO) or Acros (Geel, Belgium).
METHODS

*Animals:* Two lines of inbred male rats, DA and COP, were used. These rats were obtained from the breeding colony maintained by Dr. George Cicila at the University of Toledo Health Science Campus. They were divided into 1) sedentary control and 2) training groups matched based on body weight. Rats were weighed daily. The rats in the training group were fed ad libitum while the rats in the control group were restricted to an appropriately allotted amount of food in order to maintain comparable body weights between control and training groups throughout the experimental period. All rats were fed normal rodent chow (Ralston Purina, diet 5001).

*Swim Training:* The rats in the training group were acclimatized to swimming for 20 min one day prior to the start of swim training. The rats then swam in a bucket that contained water of approximately 50 cm in depth maintained at approximately 35°C for two 3-hour long bouts separated by a 45-min long rest during which food was given. The water temperature was measured periodically using a thermometer and maintained at 35°C by replacing water in the bucket with fresh warm water. The rats were trained in groups of 5-7 rats/group, 6 days/week, for 4 weeks. At the end of the 4 weeks of training, rats in both the control and training groups underwent glucose tolerance tests. The rats in the training groups were trained for an additional 4 days before harvesting appropriate tissues.

*Glucose Tolerance Test (GTT):* At the conclusion of the swim training regimen and, following an overnight fast, rats in both control and trained groups underwent glucose tolerance tests. The rats were put under light anesthesia by administering pentobarbital (20-40 mg/kg BW) and placed in a Plexiglas holder. Additional doses of
pentobarbital were given if needed. After the rat tail was warmed in a water bath for 10 min to dilate the tail vein, a 22 gauge catheter was inserted. Blood samples were collected before and 5, 10, 20, 50 and 75 min after i.p. glucose injection (2g/kg BW). The blood glucose level was read immediately at each time point using an Accu-Check Advantage automatic glucometer (Roche Diagnostics Corporation, Indianapolis, IN). Approximately 100 µl of blood was collected at each specified time point. Between each measurement, the rats were placed on a warm heating pad (37°C) to maintain the body temperature of the rats. The catheter was kept open by filling the catheter with approximately 10 µl of diluted heparin (0.5 U/ml).

**Tissue Collection:** Following an overnight semi-fast in which the rats were fed 50% of their usual food intake, tissue from both control and training groups under appropriate anesthesia (50 mg/kg BW) were harvested. Skeletal muscles (soleus, gastrocnemius and epitrochlearis) and liver tissues were collected, frozen with a metal tong cooled in liquid nitrogen and stored at 80°C for later analysis of the tissues.

**Biochemical analysis**

*Insulin/C-peptide Analysis:* Plasma insulin and C-peptide levels were measured according to the manufacturer’s instructions, using the rat insulin and C-peptide radioimmunoassay (RIA) kits (LINCO Research Inc, St. Charles, MO.)

*CEACAM1 Analysis by Western Blotting:* Frozen liver samples were weighed (150-200 µg) and homogenized in cold lysis buffer (1 ml) containing 150 mM NaCl, 50 mM HEPES, 0.02% Azide, 1% Triton, 1% PMSF and protease inhibitors (pH 7.6). After homogenization, sample homogenates were spun at 14,000 RPM at 4°C for 30 min and
the supernatant was collected and used as the concentrated liver lysate. 30 μg lysates from frozen tissues were analyzed directly by SDS-PAGE followed by sequential immunoblotting with their antibodies and then with monoclonal α-actin (Sigma-Aldrich) to normalize for the amount of proteins loaded on the gel. Autoradiograms were scanned for band density measurement versus that of actin and expressed as arbitrary units. CEACAM1 in liver lysates were analyzed using a polyclonal antibody against rat CEACAM1 (α-rat CC1).

**Citrate Synthase Analysis:** A fluorometric enzyme assay was employed to analyze citrate synthase activity to measure changes in oxidative capacity in skeletal muscle according to methods described by others (Chi, Hintz et al. 1983). Muscle samples were weighed while frozen and homogenized in ice cold buffer (1:50, wt/vol) containing 25% glycerol, 20 mM sodium phosphate buffer (16 mM Na₂HPO₄ + 4 mM NaH₂PO₄, pH 7.4), 5 mM beta-mercaptoethanol, 0.5 mM EDTA and 0.02% BSA. The homogenized samples were frozen at -80°C until the time of assay. At the time of the assay, the samples were diluted (1:40) in a solution containing 20 mM imidazole-HCl (pH 7.0) and 0.02% BSA. A citrate standard curve was used to determine appropriate citrate concentration range to be measured in the muscle samples. The standard curve ranged from 0 mM to 1.0 mM citrate. The assay, as previously described (Passonneau 1993), was carried out in 10x75 mm test tubes. Each test tube contained 50 μl of citrate synthase reagent containing 50 mM Tris-HCl (pH 8.1), 0.25% BSA, 400 μm acetyl CoA and 500 μM oxaloacetate (OAA). To initiate the reaction, 3 μl of diluted muscle sample and 5 μl of citrate standard were added to the appropriate tubes at 25°C. After 60 min, 5 ul of 0.5 N NaOH was added to each tube to stop the enzyme reaction. All tubes were then heated up at 95°C for
5 min and then cooled on ice. After 10 min of cooling, 500 µl of citrate reagent containing 100 mM Tris-HCl (pH 7.5), 100 µM ZnCl₂, 0.02% BSA, 30 µM NADH, 0.5 µg/mL MDH and 2.5 µg/mL citrate lyase was added to each tube. After 20 min at room temperature, the 20 µl of 2 N HCl was added to each tube. The tubes were incubated for 10 min at room temperature at which point 100 µl from each tube was aliquoted into fresh 10×75mm test tubes containing 1 ml of solution containing 6 N NaOH and 10 mM imidazole. The tubes then sat at room temperature for 60 min before they were read in the fluorometer to determine citrate synthase activity.

**Acetyl-CoA Carboxylase Analysis:** Liver samples were weighed while frozen and homogenized in ice cold buffer (1:10, wt/vol) containing 10 mM Tris-HCl (pH 7.5), 200 mM mannitol, 50 mM NaF, 1 mM EDTA, 10 mM beta-mercaptoethanol and protease inhibitors: aprotinin (1ml/100ml buffer), leupeptin (1mg/100ml buffer) and antitrypsin (1mg/100ml buffer). Immediately after homogenization, homogenate solutions were centrifuged for 40 min at 48,000g. The supernatant was collected and the volume was measured. Ammonium sulfate (144mg/ml supernatant) was added to the supernatants and stirred at 4°C for 30 min. The precipitate was then collected by centrifugation at 48,000g for 30 min. The pellet collected was dissolved in homogenate buffer (10% of original volume) and centrifuged at 48,000g for 20 min to remove any insoluble protein. The supernatant was collected and used to measure ACC activity according to the method described previously (Carlson and Winder 1999).

ACC activity was determined at a citrate concentration of 20 mM, as previously described (Winder and Hardie 1996), by measuring the rate of incorporation of 14C bicarbonate into malonyl CoA at 37°C. Reaction medium (190 µl) containing 50 mM
HEPES buffer (pH 7.5), 1.56 mM MgSO$_4$, 2 mM dithiothreitol, 0.25 mM acetyl-CoA, 4 mM ATP, 12.5 mM NaHCO$_3$, 40 µCi/mm mol NaH$^{14}$CO$_3$, 0.075% BSA, 20 mM citrate and 20 mM MgAc, was aliquoted into microcentrifuge tubes in which the reaction took place. The reaction was initiated by the addition of undiluted sample (10 µl) previously prepared. After 10 min of incubation at 37°C, the reaction was terminated by the addition of 5 N HCl (50 µl) to each tube. Tubes were centrifuged for 15 min before 150 µl of the reaction solution was aliquoted into scintillation vials and evaporated to dryness under a vacuum overnight. The next day, after adding 4 ml of scintillation fluid to each tube, radioactivity in each tube was counted using a scintillation counter (Beckman Coulter Inc., Fullerton, CA) and ACC activity in each sample was determined based on sample radioactivity and the specific activity in the incubated medium.

Triglyceride Analysis: Muscle and liver triglyceride levels were assayed in frozen tissues. Frozen tissue was weighed and placed in glass tubes containing 350 µl ethanolic KOH (2 parts EtOH: 1 part 30% KOH) (Salmon and Flatt 1985; Norris, Chen et al. 2003). A sample blank tube was set up containing 100 µl of distilled water in 350 µl ethanolic KOH. A standard triglyceride tube and a triglyceride blank tube were also set up, containing 100 µl of 4 mM glyceryl trioleate (diluted in chloroform) in 350 µl ethanolic KOH and 100 µl chloroform in 350 µl ethanolic KOH, respectively. These tubes were incubated overnight at 55°C, vortexed early in incubation, in order to hydrolyze the triglyceride into glycerol and fatty acids. Blood plasma triglycerides were hydrolyzed by adding 10 µl of plasma to 35 µl of ethanolic KOH, or 10 µl distilled water to 35 µl of ethanolic KOH as the sample blank, and incubated for 30 min at 70°C. A standard triglyceride tube and a triglyceride blank were also set up for use in the blood.
plasma assay, containing 10 μl of 4 mM glyceryl trioleate (diluted in chloroform) in 35 μl ethanolic KOH and 10 μl chloroform in 35 μl ethanolic KOH, respectively. These tubes were also incubated for 30 min at 70°C. After overnight incubation of tissue samples, the digested tissue, hydrolyzed glyceryl trioleate solution and chloroform solution were transferred to microcentrifuge tubes and their volumes were brought to 1000 μl with 50% ethanol. The tubes were centrifuged for 5 min and the supernatant was collected. The collected supernatant was brought to 1200 μl with 50% ethanol and vortexed. 500 μl of this solution was aliquoted to a fresh tube and neutralized to pH 7.0 with 3 M perchloric acid and 2 M KHCO₃. Following 30 min incubation at 70°C, diluted ethanol was not added to any tubes containing blood plasma (final volume approximately 45 μl), blood plasma sample blanks, blood plasma triglyceride standards or blood plasma triglyceride standard blanks; these tubes were neutralized as they were. After 10 min on ice, all tubes were centrifuged. The supernatant was collected and used in the assay.

Glycerol was measured fluorometrically, as previously described (Wieland 1974; Passonneau 1993). Glycerophosphate is formed from glycerol by the addition of ATP and glycerokinase (GK). Newly formed glycerophosphate is oxidized by NAD-dependent glycerophosphate dehydrogenase (GDH), forming NADH. This formation of NADH is measured fluorometrically and is proportional and indicative of the amount of glycerol present. 3 μl (2x diluted) tissue sample, tissue sample blank or 5 μl (0x diluted) plasma sample, plasma sample blank was added to reaction medium (1.1mL) containing 690 mM hydrazine (pH 9.2), 137 mM glycine, 200 μM NAD+, 0.34U/mL(2 μg/mL) GDH from rabbit muscle, 1.37 mM MgCl₂ and 1.23 mM ATP. GK (0.086U/tube) was added and reaction tubes were incubated at room temperature for 90 min before measuring glycerol.
Plasma Free Fatty Acid Analysis: Plasma free fatty acids were measured, according to the manufacturer’s instructions, in triplicate using the Wako NEFA-C assay kit (Richmond, VA).

Glycogen Analysis: Glycogen levels were assayed fluorometrically, as previously described (Passonneau 1993). This assay relies on the breakdown of glycogen to glucose via amyloglucosidase. Glucose is then converted to glucose-6-P and ADP by hexokinase in the presence of ATP. After addition of glucose-6-phosphate dehydrogenase (GDH), glucose-6-phosphate and NADP⁺, consequently are then converted to 6-P-gluconolactone and NADPH, the final product which is measured. Any changes made in protocol were done to optimize assay conditions.

Muscle tissues were ground in 3M perchloric acid (100 µl) and ddH₂O (900 µl). Two tubes were set up per sample, one containing amyloglucosidase (+AG) and the other without amyloglucosidase (-AG). 10 µl undiluted muscle homogenate or 10 µl liver homogenate (5x diluted in 0.3M perchloric acid) were added to 500 µl buffer containing 50 mM sodium acetate buffer (pH 5.5) and 0.02% BSA. Following three hours of incubation at 25°C, glucose reagent (500 µl) containing 100mM Tris-HCl (pH 8.1), 0.04% BSA, 100 µM NADP, 0.6 mM DTT, 1mM ATP, 2mM MgCl₂, 0.5U/mL glucose-6-phosphate dehydrogenase and 0.4U/mL hexokinase was added to each tube and vortexed. Following a 30-min incubation period at room temperature, tubes were read in the fluorometer to attain glucose readings for the tubes lacking amyloglucosidase. Tubes containing amyloglucosidase measured both, glucose and glycogen. Glycogen levels were determined by taking the difference of the tubes, +AG and –AG, for the same sample.
Statistical Analysis: A 2 (strain) x 2 (training status) multivariate analysis of variance (MANOVA) test was done to determine the presence of statistical difference for the two main effects (strain and training status) and the interaction between strain and training status for insulin, C-peptide and glucose tolerance curves. If a significant difference was found in the MANOVA analysis, each variable was further analyzed using analysis of variance (ANOVA). ANOVA tests were used to analyze train* training status interactions and main effects: strain and training status for variables: triglycerides, free fatty acids, glycogen, and citrate synthase. If only a main effect was significantly relevant, a t-test was employed to further characterize the main effect. Statistical significance was determined at $p \leq 0.05$. 
RESULTS

Glucose Tolerance Improvement in Both DA and COP Rat Strains Following Swim Training—To assess the effect of the exercise training on glucose tolerance, rats in both control and trained groups in both strains underwent a glucose tolerance test following completion of the swim training regimen. Glucose values were taken before and at 5, 10, 20, 50 and 75 min after i.p. glucose (2 g/kg BW) injection. Glucose tolerance was quantified by calculating the area under curve using the trapezoid rule (Tai 1994). A 58% improvement in glucose tolerance (p<0.001) was seen in trained DA rats (Figure 2) compared to a 25% improvement (p<0.01) in trained COP rats (Figure 3). There was no difference in the level of glucose tolerance between the two strains in the control groups, suggesting that the level of an intrinsic glucose tolerance was same in the two strains. However, there was a significant difference (p<0.05) in glucose tolerance level in the two trained groups between the two strains, showing better glucose tolerance in DA than in COP. These results suggest that indeed, swim training does result in glucose tolerance improvement, indicative of increased insulin sensitivity and that genetic difference does influence the degree of glucose metabolic adaptation to exercise training.
Figure 2: The results of glucose tolerance test in DA rats. Glucose tolerance tests were administered to evaluate the influence of 4 weeks of swim training on the level of glucose tolerance in the DA animals. Glucose readings were taken before and 5, 10, 20, 50 and 75 min after i.p. glucose (2g/kg BW) injection for both control (n=8) and trained (n=7) rats. A 58% improvement in glucose tolerance was seen in response to training in DA animals. *p<0.05, **p<0.01, and ***p<0.001 vs. control at corresponding time or AUC in control.
Figure 3: The results of glucose tolerance in COP rats

Figure 3: The result of glucose tolerance test in COP rats. Glucose tolerance tests were administered to evaluate the influence of 4 weeks of swim training on the level of glucose tolerance in the COP animals. Glucose readings were taken before and 5, 10, 20, 50 and 75 min after i.p. glucose (2g/kg BW) injection for both control (n=6) and trained (n=7) rats. A 25% improvement in glucose tolerance was seen in response to training in COP animals. *p<0.05, **p<0.01, and ***p<0.001 vs. control at corresponding time or AUC in control.

Plasma Insulin Levels in Both DA and COP Rats Following Swim Training—To evaluate whether improved glucose tolerance observed does in fact result in improved insulin sensitivity and beta cell function, plasma insulin levels were measured before and
5, 10, 20, 50 and 75 min after i.p. glucose (2 g/kg BW) injection. The results are shown in Fig. 4 for DA and Fig. 5 for COP. There was no significant difference in the area under the curve between the control and training groups in DA rats (11280.95 ± 1449.85 pM in control vs. 17835.39 ± 3238.56 pM in trained group; Figure 4). Similar findings were observed in COP rats, with no significant difference in the area under the curve between control and training groups (18955.42 ± 2543.59 pM in control vs. 19835.10 ± 2133.48 pM in trained group; Figure 5). Contrary to what was expected, based on data reported by others (Reitman, Vasquez et al. 1984) a trend of increased insulin levels in the area under the curve in the trained group compared to the control group in both strains was observed. Further analysis was done on basal insulin values using ANOVA test to determine whether training elicited a change in insulin sensitivity in the basal state. There was a significant main effect of training status (p<0.01) without a significant effect of strain or of the interaction between training status and strain (Figure 6). To locate a difference between control and trained groups, a t-test was conducted on each strain and we found that basal insulin levels in the DA trained group were significantly lower (p<0.05) than that in the DA control group while apparent trends were observed, without significant difference in basal insulin levels in response to training in COP rats.

These results suggest that training does result in decreased fasting insulin levels in trained animals, an indication of improved insulin sensitivity. However, these results also show that training has likely caused an increased response by the pancreatic beta cells resulting in greater insulin secretion in response to glucose, an indication of increased beta cell sensitivity.
Figure 4: The results of plasma insulin levels in DA rats.

Figure 4: The results of plasma insulin levels in DA rats. Insulin levels were assayed in control (n=8) and trained (n=7) DA animals using Insulin RIA kits to determine whether increased glucose tolerance, in response to training, seen in the glucose tolerance tests was also accompanied by improved insulin sensitivity. Insulin levels were assayed on blood samples obtained before and after 5, 10, 20, 50 and 75 minutes after i.p. glucose (2 g/kg BW) injection. The area under the curve calculated was 11280.95 ± 1449.85 for control rats and 17835.39 ± 3238.56 for trained rats, revealing insignificant trends of increased secretion of insulin in response to swim training.
**Figure 5: The results of plasma insulin levels in COP rats**

Insulin levels were assayed in control (n=6) and trained (n=7) COP animals using Insulin RIA kits to determine whether increased glucose tolerance, in response to training, seen in the glucose tolerance tests was also accompanied by improved insulin sensitivity. Insulin levels were assayed on blood samples obtained before and after 5, 10, 20, 50 and 75 minutes after i.p. glucose (2 g/kg BW) injection. The area under the curve calculated was 18955.42 ± 2543.59 for control rats and 19835.10 ± 2133.48 for trained rats, revealing no significant difference in insulin secretion as a result of swim training.
Figure 6: The results of basal insulin levels in COP and DA rats

![Bar graph showing basal insulin levels in COP and DA rats.](image)

**Figure 6: The results of basal insulin levels in COP and DA rats.** Basal insulin levels shown in Graph 4 and 5 are redrawn in a bar graph for the clarity of presentation. ANOVA results showed a significant effect of training status (p < 0.05) without the effect of strain or the interaction between strain and training status. * p < 0.05 vs. control.

**C-Peptide Levels in Both the DA and COP Strains Following Swim Training**—To further evaluate insulin secretion, C-peptide levels were measured from blood plasma sample obtained before and at 5, 10, 20, 50 and 75 min after i.p. glucose (2g/kg BW) injection. The results are shown in Fig. 7 for DA rats and in Fig. 8 for COP rats. There
was no significant difference found in the area under the curve between the control and trained groups in DA animals (80173.79 ± 7268.37 pM in control vs. 85885.88 ± 10312.35 pM in the trained group; Figure 7). We observed similar results in COP rats, with no significant difference in the area under the curve between control and trained groups (129098.31 ± 14319.19 pM in control vs. 107542.81 ± 9407.48 pM in the trained group; Figure 8). It is noteworthy to mention that the patterns of C-peptide curves in both strains resemble those of insulin curves shown Fig. 4 and Fig. 5. As shown in the insulin curve, there was a trend of an increase in the area under the curve in the trained group compared to the control group in DA rats.

As done in insulin levels in basal state, an ANOVA test was conducted on basal C-peptide levels to determine whether the findings of improvement in insulin sensitivity after the swim training could be also observed in changes in C-peptide in basal state. There was a significant main effect of training status (p<0.01) without the significant effect of strain or of the interaction between training status and strain (Figure 6). To locate a difference between control and trained groups a t-test was conducted on each strain and it was found that basal C-peptide levels in the trained group in DA were significantly lower than that in the control (291.45 ± 27.05 pM in trained vs. 632.11 ± 57.14 pM in the control group; p<0.05) while there was no significant difference in COP strain though C-peptide level in the trained group did show less than that in the control group (381.74 ± 68.40 pM in trained vs. 519.63 ± 115.34 pM in the control group; p>0.05).
Figure 7. The results of C-peptide levels in DA rats. C-peptide levels were assayed in control (n=8) and trained (n=7) DA animals using C-peptide RIA kits to determine whether increased glucose tolerance, in response to training, seen in the glucose tolerance tests was also accompanied by improved insulin sensitivity. C-peptide levels were assayed on blood samples obtained before and after 5, 10, 20, 50 and 75 minutes after i.p. glucose (2 g/kg BW) injection. The area under the curve calculated was 80173.79 ± 7268.37 for control rats and 85885.88 ± 10312.35 for trained rats, revealing insignificant trends of increased secretion of C-peptide in response to swim training.
Figure 8: The results of C-peptide levels in COP rats. C-peptide levels were assayed in control (n=6) and trained (n=7) COP animals using C-peptide RIA kits to determine whether increased glucose tolerance, in response to training, seen in the glucose tolerance tests was also accompanied by improved insulin sensitivity. C-peptide levels were assayed on blood samples obtained before and after 5, 10, 20, 50 and 75 minutes after i.p. glucose (2 g/kg BW) injection. The area under the curve calculated was 129098.31 ± 14319.19 for control rats and 107542.81 ± 9407.48 for trained rats, revealing no significant difference in C-peptide secretion as a result of swim training.

Hepatic CEACAM1 expression upregulated in response to swim training in DA rats— To evaluate insulin clearance capacity we measured hepatic CEACAM1 expression by Western blotting. Hepatic CEACAM1 expression was measured in trained
and control groups of both DA and COP. The results are shown in Figure 9. Significant upregulation (p<0.05) of 85% in hepatic CEACAM1 expression was seen in response to 4 weeks of swim training in DA rats but not in COP rats (Figure 9). These results reiterate that genetic factors do indeed influence degree of adaptive changes in response to endurance training and that these adaptive changes can be reflected in CEACAM1 expression. Increased hepatic CEACAM1 observed in DA rats coincides with a large improvement in glucose tolerance and a significant improvement in insulin sensitivity in a basal state in these rats, suggesting that CEACAM1 protein plays an important role in increased insulin sensitivity in response to training.
Figure 9: The results of hepatic CEACAM1 expression

Figure 9: The results of hepatic CEACAM1 expression. Hepatic CEACAM1 expression of control and trained animals in both DA and COP strains was assayed by Western blot analysis to assess the capacity of insulin clearance and any adaptive change in expression in response to 4 weeks of endurance training. A representative gel is pictured, revealing a significant upregulation (85%, P<0.05) in hepatic CEACAM1 expression in DA trained (n=3) animals versus untrained (n=3). There is no significant difference in CEACAM1 expression between the COP trained (n=3) and the COP untrained (n=4) rats. *p<0.05 vs. untrained in DA.

Citrate Synthase Activity Elevated in Red Skeletal Muscle Fibers in Response to Swim Training—Citrate synthase activity in red and white gastrocnemius, soleus and epitrochlearis was fluorometrically assayed to assess adaptive changes in oxidative capacity in response to 4 weeks of swim training. The results are shown in Table I. Following the endurance training regimen, significant increase in citrate synthase activity
was observed in red gastrocnemius muscle in both DA (36%) and COP (42%) rats (Table I). Significant increase in citrate synthase activity was also observed in soleus muscle in both DA (59%) and COP (46%) rats (Table I). However, significant increase in citrate synthase activity in white gastrocnemius muscle was observed only in COP (47%) rats not in DA rats. No significant change was observed in citrate synthase activity in epitrochlearis muscle in either strain. In general, no big difference was observed in adaptive changes in citrate synthase activity in skeletal muscles between the two strains. Based on adaptive changes in citrate synthase activity it appears that both strains of rats recruited expected pattern of muscle motor units during swim training, that is, slow-oxidative fibers (soleus), fast-oxidative fibers (red gastrocnemius) and fast-glycolytic fibers (white gastrocnemius).
Table I: Citrate synthase activity in skeletal muscles

<table>
<thead>
<tr>
<th>Muscles</th>
<th>DA control</th>
<th>DA trained</th>
<th>COP control</th>
<th>COP trained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red Gastrocnemius</td>
<td>28.57 ± 2.15</td>
<td>38.73 ± 2.88**</td>
<td>22.77 ± 1.69</td>
<td>32.38 ± 0.96***</td>
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<td>Soleus</td>
<td>25.59 ± 1.42</td>
<td>40.73 ± 2.47***</td>
<td>26.25 ± 1.86</td>
<td>37.25 ± 2.97**</td>
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<tr>
<td>White Gastrocnemius</td>
<td>13.60 ± 1.75</td>
<td>14.53 ± 1.67</td>
<td>12.24 ± 1.03</td>
<td>17.96 ± 1.94*</td>
</tr>
<tr>
<td>Epitrochlearis</td>
<td>13.65 ± 1.32</td>
<td>16.90 ± 2.06</td>
<td>16.04 ± 1.80</td>
<td>15.08 ± 1.44</td>
</tr>
</tbody>
</table>

Table I: Citrate synthase activity in skeletal muscles. Mitochondrial enzyme marker, citrate synthase (CS) activity, was fluorometrically assayed to assess change in oxidative capacity in red gastrocnemius, soleus, white gastrocnemius and epitrochlearis muscles in response to exercise training in both control (n=10) and trained (n=8) DA rats, and control (n=7) and trained (n=7) COP rats. Data was expressed in µmol/g/min, mean ± SEM. An increase in CS activity of 36% (p<0.01) and 42% (p<0.001) was observed in DA and COP rats, respectively in red gastrocnemius muscle. An increase in soleus CS activity of 59% (p<0.001) and 42% (p<0.01) was observed in DA and COP rats, respectively. An increase in CS activity of 47% (p<0.05) was observed in COP rats only not in DA, in white gastrocnemius muscle. No significant change in CS activity in epitrochlearis muscle was found in either rat strain. Results suggest that oxidative adaptive changes, in response to exercise, are more apparent in red muscle fibers than in white muscle fibers. *p < 0.05, **p < 0.01, and ***p < 0.001 vs. control.

Hepatic Acetyl-CoA Carboxylase(ACC) Activity Following Swim Training—

Because aberrant fat metabolism has been shown to contribute to glucose intolerance and insulin resistance, the activity of ACC, a key enzyme involved in an initial step of the lipogenic pathway, in the liver, was measured to evaluate fat metabolism efficiency. This enzyme activity was assayed by measuring the rate of incorporation of the carboxyl
group from $^{14}$C-bicarbonate into acetyl CoA which is converted into malonyl CoA. These results are shown in Figure 10. Though trends of elevated hepatic ACC activity following exercise training were observed, indicating increased levels of lipogenesis, no significant difference was found in either rat strain (Figure 10).

**Figure 10: Hepatic Acetyl-CoA Carboxylase Activity**

![Graph showing hepatic ACC activity](image)

**Figure 10: Hepatic Acetyl-CoA Carboxylase Activity.** ACC activity in liver tissue was determined at citrate concentrations of 20 mM, by measuring the rate of incorporation of the carboxyl group from $^{14}$C bicarbonate into acetyl CoA at 37°C. Liver tissue was assayed from trained (n=8) and control (n=10) DA rats, and trained (n=7) and control (n=7) COP rats. No significant difference was found in either strain in response to swim training.
Triglyceride Accumulation in Skeletal Muscle and Liver Tissue in Response to Endurance Exercise—Because elevated tissue triglycerides have been previously implicated to hinder glucose utilization (Straczkowski, Kowalska et al. 2001) and to further analyze fat metabolism in liver and skeletal muscle, triglyceride levels were assayed fluorometrically. These results are shown in Table II. The levels of triglyceride in the muscles (soleus and white gastrocnemius) were not statistically different between the controls and the trained groups in both DA and COP strains. However, there was a significant decrease (26%, p<0.05) in the liver in the trained group compared with that in the control group in COP rats but not in DA rats.
Table II: The results of tissue triglyceride levels

<table>
<thead>
<tr>
<th>Tissue</th>
<th>DA Control</th>
<th>DA Trained</th>
<th>COP Control</th>
<th>COP Trained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>15.12 ± 1.65</td>
<td>15.06 ± 1.35</td>
<td>19.92 ± 1.16</td>
<td>14.67 ± 1.08*</td>
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<tr>
<td>Soleus</td>
<td>12.53 ± 1.54</td>
<td>12.65 ± 3.54</td>
<td>13.71 ± 1.44</td>
<td>10.58 ± 0.77</td>
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<tr>
<td>Red Gastrocnemius</td>
<td>6.49 ± 0.600</td>
<td>7.37 ± 1.94</td>
<td>5.79 ± 0.92</td>
<td>8.03 ± 0.56</td>
</tr>
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</table>

Table II: The results of tissue triglyceride levels. Tissue triglyceride levels were assayed by fluorometrically measuring the amount of glycerol present as previously described (Wieland, 1974; Passonneau and Lowry, 1993). Liver tissue was assayed from trained (n=7) and control (n=8) DA rats, and trained (n=7) and control (n=7) COP rats. Soleus tissue was assayed from trained (n=7) and control (n=8) DA rats, and trained (n=5) and control (n=6) COP rats. Red gastrocnemius tissue was assayed from trained (n=7) and control (n=9) DA rats, and trained (n=7) and control (n=6) COP rats. *p < 0.05 vs. control

Plasma Triglycerides in DA and COP rats — To further assess lipid metabolism in response to endurance exercise training, plasma triglyceride levels in an overnight-fasted basal state, were assayed fluorometrically. As the results are shown in Figure 11, trained rats, both DA and COP, displayed significantly decreased levels of plasma triglycerides (p<0.05).
Figure 11: The results of plasma triglyceride levels

Figure 11: The results of plasma triglycerides. Plasma triglyceride levels were assayed by fluorometrically measuring the amount of glycerol present as previously described (Wieland, 1974; Passonneau and Lowry, 1993). Plasma was assayed from trained (n=6) and control (n=6) DA rats, and trained (n=5) and control (n=3) COP rats. *p<0.05 vs. control.

*Plasma Free Fatty Acids Following Exercise Training*— Fasting free fatty acid levels were measured in plasma to further evaluate fat metabolism at rest following endurance training (Figure 12). As results are shown in Figure 12, no significant difference between the untrained and trained groups in both DA and COP was found.
Figure 12: The results of fasting plasma free fatty acids. Plasma free fatty acids were measured using the Wako NEFA-C assay kit (Waco, TX). Plasma was assayed from trained (n=7) and control (n=8) DA rats, and trained (n=7) and control (n=6) COP rats.

Post-exercise Glycogen Levels Surpass Basal Levels— To further characterize metabolic changes occurring in response to exercise training, we measured glycogen levels in skeletal muscle and liver. These results are shown in Table III. Glycogen levels, following training, provide us with information regarding metabolic efficiency.

Significantly elevated levels of glycogen were observed post-training in COP rats in red gastrocnemius (75% increase, p<0.01), white gastrocnemius (31% increase, p<0.05), epitrochlearis (60% increase, p<0.01) and liver tissues (181% increase, p<0.001). In DA
rats, significantly increased glycogen levels are seen only in epitrochlearis muscle following training (Table III). This data suggests improved glucose metabolism efficiency in terms of glycogenesis as a consequence of exercise training, providing the body with a more abundant source of energy for its next bout of training. This improved efficiency is however, more apparent in the COP rats.
Table III: Glycogen levels

<table>
<thead>
<tr>
<th>glycogen</th>
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<th>DA Trained</th>
<th>COP Control</th>
<th>COP Trained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red Gastrocnemius</td>
<td>11.17 ± 1.49</td>
<td>16.51 ± 2.92</td>
<td>15.27 ± 2.02</td>
<td>26.66 ± 3.04**</td>
</tr>
<tr>
<td>Soleus</td>
<td>14.29 ± 1.10</td>
<td>14.70 ± 0.88</td>
<td>12.65 ± 1.06</td>
<td>13.77 ± 1.53</td>
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<tr>
<td>White Gastrocnemius</td>
<td>21.67 ± 1.61</td>
<td>26.72 ± 2.53</td>
<td>23.97 ± 1.37</td>
<td>31.39 ± 2.49*</td>
</tr>
<tr>
<td>Epitrochlearis</td>
<td>14.68 ± 1.68</td>
<td>33.73 ± 3.67***</td>
<td>22.28 ± 2.34</td>
<td>35.63 ± 2.98**</td>
</tr>
<tr>
<td>Liver</td>
<td>92.23 ± 17.96</td>
<td>124.57 ± 16.47</td>
<td>60.31 ± 10.70</td>
<td>169.68 ± 28.69**</td>
</tr>
</tbody>
</table>

µmol/g ± SEM

Table III: Glycogen levels. Glycogen levels were indirectly assayed by fluorometrically measuring glucose after hydrolysis of glycogen in the presence of amylglucosidase, as previously described (Passonneau and Lowry, 1993). Liver tissue was assayed from trained (n=8) and control (n=10) DA rats, and trained (n=7) and control (n=7) COP rats. Soleus tissue was assayed from trained (n=8) and control (n=10) DA rats, and trained (n=7) and control (n=7) COP rats. Red and white gastrocnemius tissue was assayed from trained (n=8) and control (n=10) DA rats, and trained (n=7) and control (n=7) COP rats. Epitrochlearis muscle was assayed from trained (n=8) and control (n=10) DA rats, and trained (n=7) and control (n=6) COP rats. *p< 0.05, **p< 0.01, and ***p< 0.001 vs. control.
Discussion

The goal of this study was to test the feasibility of creation of an animal genetic model by testing the inbred strains of rats for exercise training-induced improvement in glucose tolerance and to characterize metabolic intermediate phenotypes that may explain difference, if present at all, in exercise training-induced change in glucose tolerance between two inbred strains of rats.

To this end, we employed two inbred rat strains in our study, DA and COP, which are known to possess high and low intrinsic aerobic capacities, respectively (Barbato et al., 1998). Following 4 weeks of endurance swim training regimen, the rats in the control and trained group in the both strains underwent glucose tolerance tests. As shown in the results section, both strains improved their levels of glucose tolerance significantly, with greater improvement in DA (58%) than in COP (25%; see Figures 2 and 3). It is often observed that exercise training-induced improvement in metabolism depends on an initial level prior to the onset of training (Atalay, Oksala et al. 2004). However, in this study there was no difference in intrinsic (basal) glucose tolerance between the two strains observed in the rats in the control groups. Thus, one can rule out the effect of intrinsic glucose tolerance on a large divergent response in glucose tolerance between DA and COP strains in response to exercise training. Since these rats had been bred under identical environmental conditions such as diet and temperature, it is expected that difference in other environmental factors between these two strains are minimal. It is likely that the large difference in training-induced improvement between DA and COP is due to differences in genetic factors between these strains of rats.
To determine what factors may account for the observed difference seen in improvement of glucose tolerance after exercise training between both strains, we investigated a number of potentially influential intermediate phenotypes. Insulin sensitivity is known to increase as a result of exercise training with a blunted response of insulin to glucose challenge, indicative of a more efficient response to insulin (Wojtaszewski, Nielsen et al. 2002). Contrary to this expectation, our data shows a trend of an increase in insulin secretion, particularly in DA rats (see Fig. 4 and 7), though statistically insignificant, during the Glucose Tolerance Test, from the pancreas in response to training (Figures 4, 5, 7). We are unaware of any previous investigators who have observed such an unusual response of insulin during glucose tolerance test in these inbred rats after exercise training. However, exercise training caused improvement in insulin sensitivity in the basal state in DA rats, as we found a significant decrease (p<0.05) in insulin level in the trained rats compared with that in the control group. This trend was also seen in COP rats (Fig. 6). It is not known what may have caused such unusual insulin responses in these rats. However, it is worth mentioning, that these trends of unexpected increased insulin secretion corresponded with trends of elevated basal state plasma free fatty acid levels (Figure 12) in both DA and COP rats. Long-term exposure of the pancreatic β-cell to free fatty acids has been previously shown to result in hypersensitivity of the cells, causing increased insulin secretion in response to glucose (Liu, Tornheim et al. 1998). Therefore it is reasonable to conclude that trends of increased insulin secretion by the β-cell, observed in both DA and COP, may be due in part to long-term free fatty acid exposure.
Triglycerides comprise the largest energy store in the body (Horowitz and Klein 2000). These triglycerides are located in adipose tissue, skeletal muscle, the liver and in blood plasma. In response to energy demands, triglycerides are hydrolyzed into one glycerol and 3 fatty acids, through a process called lipolysis (Horowitz and Klein 2000). Free fatty acids are then taken up by skeletal muscle and oxidized in the mitochondria for ATP (energy) generation. Because fatty acid oxidation provides the maximum amount of energy required for endurance exercise, TG hydrolysis in adipose tissue, skeletal muscles and plasma is necessary to provide adequate amounts of free fatty acids (Horowitz and Klein 2000)). Increased lipolysis (TG hydrolysis), in response to exercise, as indicated by significantly decreased levels of plasma triglycerides, shown in Figure 11(p<0.05), and trends of elevated levels of plasma free fatty acids (Figure 12) demonstrate increased availability of free fatty acids for subsequent increased oxidation to meet energy requirements. These results were similar for both DA and COP strains and thus do not provide us with an intermediate phenotype to account for the difference seen in glucose tolerance.

Prior studies have shown a negative correlation between hepatic TG content and insulin-induced suppression of hepatic glucose production (Ryysy, Hakkinen et al. 2000; Seppala-Lindroos, Vehkavaara et al. 2002), implicating that hepatic lipid accumulation may induce hepatic insulin resistance. It has also been previously demonstrated that TG accumulation in skeletal muscle is associated with insulin resistance and T2DM (Storlien, Jenkins et al. 1991; Pan, Lillioja et al. 1997; Perseghin, Scifo et al. 1999). We, therefore, investigated levels of TG in the liver and in various skeletal muscles to determine
whether TG accumulation in these tissues may account for the difference in training-induced improvement in glucose tolerance. Following exercise training, DA rats demonstrated no significant change in hepatic TG levels (Table II), however, COP rats demonstrated a significant (p<0.05) decrease in hepatic TG levels (Table II). This difference in hepatic adaptation between the two strains may contribute to observed differences in glucose tolerance following training. However, neither strain showed a significant difference in TG accumulation, in response to exercise training, in either soleus or red gastrocnemius muscle (Table II). Our results suggest that these muscles, in both strains, adapt similarly to a swim training regimen and therefore cannot account for the differences seen in glucose tolerance.

Another element of fat metabolism investigated in our study was fatty acid synthesis. Lipogenic capacity in the liver was measured by assaying for ACC activity. ACC facilitates malonyl CoA generation, a known inhibitor of fatty acid oxidation in the liver. The major isoform of ACC expressed in the liver is ACCα, which is activated by citrate and inactivated by AMPK (Carlson and Winder 1999). It has been previously shown that ACC activity and malonyl CoA content in rat liver and skeletal muscle decreased, immediately following treadmill running (Carlson and Winder 1999). We assessed the ACC activity in the presence of 20 mM of citrate, which causes the maximal activation of the enzyme to assess the maximal capacity of lipogenic capacity in the liver in basal state (Winder and Hardie 1996). Though we saw trends of increased ACC activity in response to exercise, we found no statistical significant difference in ACC activity between the control and the trained rats in the either rat strain (Figure 10). This suggests that the protein expression of ACC is not inducible by aerobic exercise training.
and rather is modulated with covalent modification by AMPK. In future studies, we plan to further assess the lipogenic enzymes activity in the liver and skeletal muscle harvested immediately after exercise, as others have previously done (Rasmussen and Winder 1997; Ruderman, Saha et al. 1999).

To evaluate possible adaptation of the oxidative pathway of metabolism in response to swim training, we assayed for citrate synthase (CS), a major regulatory enzyme in the citric acid cycle (Siu, Donley et al. 2003). Increased CS activity and other enzymes associated with aerobic metabolism have been shown to coincide with low activity levels of glycogenolytic enzymes in humans (Chi, Hintz et al. 1983). An increase in CS activity in skeletal muscles following training in both strains, excluding epitrochlearis, indicates an increase in oxidative capability (Table I). In our experiments we found that this increase in oxidative capacity was most apparent in slow-twitch red fibers, fibers known to possess higher respiratory capacity than white fibers (Chi, Hintz et al. 1983). There was a minimal change in oxidative capacity in white muscles in both strains (Table I). However, we found a large increase in glycogen accumulation in epitrochlearis muscle in both strains and white gastrocnemius in COP strain (Table 3). These results are consistent with those found in studies conducted by Chi and associates (Chi, Hintz et al. 1983). Glycogen availability is especially important for muscles relying on glycogen catabolism as a major energy source for contractility (Wojtaszewski, Nielsen et al. 2002). Glycogen supercompensation following a state of glycogen depletion, such as exercise, is therefore evident in such muscles as were seen in our results (Table III). Our data suggests that metabolic pathways for energy derivation differ depending on the muscle fiber type in response to exercise. We found that red slow-twitch fibers rely
predominantly on aerobic carbohydrate metabolism (Table I) whereas white fast-twitch fibers depend more on glycogen catabolism for energy in both rat strains (Table III). However in COP rats, not only do we see hepatic glycogen buildup following exercise but we also see glycogen accumulation in both fast and slow twitch muscles (Table III) suggesting a difference in degree of hepatic and skeletal muscle adaptation from DA rats. Because of its insulin clearance capacity, another feature of glucose tolerance lies in the expression of CEACAM1. CEACAM1 expression has not been previously characterized in response to exercise training. Our results, shown in Figure 9, demonstrate a significant increase in its expression (85%) in response to swim training in DA rats, but not in COP rats. It is clear that adaptation to exercise varies between DA and COP strains in terms of CEACAM1. Differences in hepatic adaptation in terms of triglyceride levels (Table II), glycogen levels (Table III) and CEACAM1 expression (Figure 9), in response to exercise between the two rat strains is evident. Improvement in glucose tolerance is observed in both strains, but more so in DA than in COP. According to our results, hepatic adaptation in response to training, is clearly reflected in CEACAM1 expression in DA rats, however, hepatic adaptation in COP rats lies in triglyceride (Table II) and glycogen (Table III) levels. These apparent differences in hepatic adaptation may contribute to differences seen in glucose tolerance between DA and COP strains. However, among these changes, it is not known which variable may affect glucose tolerance the most. Because improvement in glucose tolerance is greater in DA rats (Figure 2), we can deduce that the effects of an increase in hepatic CEACAM1 expression in the DA strain (Figure 9) outweighs the effects of decreased levels of hepatic TG (Table II) and increased levels of hepatic glycogen (Table III) in the COP strain, in response to training. Therefore, we can
conclude that an element in the difference in glucose tolerance observed between the strains is hepatic CEACAM1 expression
Conclusion

To determine whether training induced adaptive change in glucose tolerance was due in part to genetic factors, we investigated two different strains of rats, Copenhagen (COP) and DA. DA rats have been previously characterized to possess a twofold greater intrinsic capacity for endurance treadmill training than COP rats, providing genetic models representing both high and low aerobic capacities (Barbato, Koch et al. 1998; Koch, Green et al. 2005). Rats from both strains were divided into 1) sedentary control and 2) training groups. They then swam in a bucket that contained water of approximately 50 cm in depth maintained at approximately 35°C for two 3-hour long bouts separated by a 45-min long rest during which food was given. At the end of the 4 weeks of training, rats in both the control and training groups underwent glucose tolerance tests. An improvement in glucose tolerance was observed in both strains following training, however a greater improvement was demonstrated by DA rats (Figure 2).

Potential metabolic intermediate phenotypes were examined in order to determine what was responsible for difference seen in glucose tolerance between the two strains. Intermediate phenotypes were chosen for evaluation based on their involvement in glucose metabolism. Though we saw trends of increased insulin (Figures 4 and 5) and C-peptide (Figures 7 and 8) levels in response to training, differences in training-induced improvement in glucose tolerance do not appear to be a result of varied insulin or C-peptide levels because we see no difference between the two strains.

A training effect was observed in citrate synthase activity in predominantly red slow-twitch skeletal muscle fibers in both DA and COP rats (Table I). However, no
significant difference was seen in training-induced oxidative change between strains, thus this intermediate phenotype cannot account for difference in glucose tolerance.

Neither training status nor strain difference affected skeletal muscle triglyceride stores (Table II). However, a training effect was evident in hepatic triglyceride build up in COP rats (Table II). Training also resulted in significantly decreased plasma triglyceride levels (Figure 11) in both rat strains, indicative of both increased plasma triglyceride hydrolysis to generate greater amounts of free fatty acids (Figure 12) for subsequent β-oxidation in response to increased energy demands (Horowitz and Klein 2000). However, no significant difference was observed in either plasma triglyceride or free fatty acid levels between strains in terms of training-induced adaptation, therefore these intermediate phenotypes cannot explain glucose tolerance variation. Neither training effect or strain difference played a role in acetyl-CoA carboxylase activity in hepatic lipogenesis, thus this enzyme does not provide explanation for difference in glucose tolerance.

Training effects were seen in both DA and COP strains in terms of glycogen synthesis in the post-exercised state (Table III). However, training effects on hepatic glycogen synthesis were more evident in COP rats. A significant (p<0.05) training effect was observed in expression of hepatic CEACAM1 in DA rats only (Figure 9). We found an 85% increase in training-induced hepatic CEACAM1 expression in DA rats. Differences in hepatic adaptation between strains in terms of CEACAM1 expression, levels of triglycerides and levels of glycogen may all contribute to differences observed in training-induced glucose tolerance. Because DA rats had a greater improvement in glucose tolerance in response to exercise than COP rats, we may deduce that increased
hepatic CEACAM1 expression in DA rats may account for difference in training-induced glucose tolerance between strains.
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Abstract

To determine the effect of genetic factors on training-induced change in glucose tolerance, Copenhagen (COP) and DA rats were studied. After 4 weeks of endurance swim training, an improvement in glucose tolerance was assessed by performing glucose tolerance tests on trained and untrained rats. Intermediate metabolites in tissues and blood were also investigated. DA showed a greater improvement (P<0.05) in glucose tolerance than did COP in response to training. There were no differences in adaptive changes in plasma insulin and C-peptide, muscle oxidative capacity, lipogenic capacity in the liver, blood lipids, or muscle TG between the two strains in response to training. Among variables (hepatic glycogen, triglyceride, and CEACAM1 expression) that showed difference between the two strains, increased hepatic CEACAM1 expression in DA coincided with a large improvement in training-induced glucose tolerance in DA rats, implicating it as the most influential factor on the strain difference seen in glucose tolerance.