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# Congenic strains confirm aerobic running capacity quantitative trait loci on rat chromosome 16 and identify possible intermediate phenotypes

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**Ways JA, Smith BM, Barbato JC, Ramdath RS, Pettee KM, DeRaedt SJ, Allison DC, Koch LG, Lee SJ, Cicila GT.** Congenic strains confirm aerobic running capacity quantitative trait loci on rat chromosome 16 and identify possible intermediate phenotypes. *Physiol Genomics* 29: 91–97, 2007. First published December 19, 2006; doi:10.1152/physiolgenomics.00027.2006.—We previously identified two inbred rat strains divergent for treadmill aerobic running capacity (ARC), the low-performing Copenhagen (COP) and the high-performing DA rats, and used an $F_2$(COP×DA) population to identify ARC quantitative trait loci (QTLs) on rat chromosome 16 (RNO16) and the proximal portion of rat chromosome 3 (RNO3). Two congenic rat strains were bred to further investigate these ARC QTLs by introgressing RNO16 and the proximal portion of RNO3 from DA rats into the genetic background of COP rats and were named COP.DA(chr 16) and COP.DA(chr 3), respectively. COP.DA(chr 16) rats had significantly greater ARC compared with COP rats (696.7 ± 38.2 m vs. 571.9 ± 27.5 m, $P = 0.03$). COP.DA(chr 3) rats had increased, although not significant, ARC compared with COP rats (643.6 ± 40.9 m vs. 571.9 ± 27.5 m). COP.DA(chr 16) rats had significantly greater subcutaneous abdominal fat, as well as decreased fasting triglyceride levels, compared with COP rats ($P < 0.05$), indicating that genes responsible for strain differences in fat metabolism are also located on RNO16. While this colocalization of QTLs may be coincidental, it is also possible that these differences in energy balance may be associated with the superior running performance of COP.DA(chr 16) consomic rats.

treadmill endurance test; abdominal fat; subcutaneous abdominal fat; consomic; triglycerides

TREADMILL EXERCISE TESTS MEASURE the integrative capability of multiple physiological systems to influence the overall adaptation to a bout of exercise and are often used to assess overall health and predict mortality (13, 22, 30). The greater the functional capacity of each system, the more efficiently an individual will adapt to the exercise, leading to a greater aerobic performance. Similarly, systems with a diminished functional capacity will be less capable of adapting to exercise stress, leading to decreased aerobic performance, and may reflect an increased susceptibility to disease development.

Treadmill running capacity is a complex trait where the interactions of multiple genetic and environmental factors influence overall performance (5). The genetic component explains a large amount of variation in performance within a given population, with human and rodent studies estimating the heritability of exercise performance to range from 39.0 to 73.0% (4, 19, 24, 25). This genetic component likely results from a complex mixture of multiple genes, with each exerting relatively minor effects on performance (2–4, 15, 19, 24, 25). We may be able to improve the likelihood of observing effects from major genes if we dissect this running performance trait into simpler, less complex, phenotypes. This would facilitate identification of genes regulating aerobic running capacity (ARC) as well as the molecular pathways through which they function.

Congenic and consomic strains have proven useful in confirming and delimiting the locations of quantitative trait loci (QTLs), as well as reducing the complexity of genetic models (16, 21). While many phenotypic differences identified in comparisons of congenic strains with their parental inbred strains will be unrelated to the QTL of interest, such differences can be used in conjunction with subsequently developed congenic substrains to determine whether they colocalize to smaller chromosomal intervals. Such an approach linked differences in an intermediate phenotype (adrenal capacity to synthesize 18-hydroxydeoxycorticosterone) with both a chromosome 7 blood pressure QTL in Dahl rats and a candidate gene (*Cyp11b1*) whose protein shows strain differences in both sequence and activity (9, 10).

Using a segregating population bred from Copenhagen (COP) and DA rats, we previously identified two quantitative trait loci (QTLs) for ARC on rat chromosome 16 (RNO16), and a suggestive QTL for ARC on rat chromosome 3 (RNO3) (39). In the above-mentioned genome scan we also observed an interaction between loci in two intervals carrying ARC QTLs, *D16Rat55* and *D3Rat56*, such that at least one DA rat allele was needed at each locus for a greater best distance run to exhaustion (39). Genes involved in lipid metabolism were identified near the ARC logarithm of odds ratio (LOD)-plot peaks as potential candidates to explain the strain differences in ARC. Furthermore, Lee et al. (23) identified genes associated with energy expenditure that were differentially expressed in the left ventricles of DA and COP rats.

The present study was performed to confirm whether the chromosomal regions identified in the $F_2$(COP×DA) genome scan influence treadmill running performance. Congenic strains were developed by introgressing alleles from the ARC QTL-containing regions of the high-performing strain (DA) onto a low-performing background (COP). We sought to further characterize these strains using anatomical and physiological measurements as means to identify potential intermediate phenotypes related to energy expenditure that may

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aid in identifying the underlying genes responsible for heritable strain differences in ARC. One of these congenic strains [COP.DA(chr 16)] performed significantly greater compared with the parental, COP strain, confirming the presence of an ARC QTL on RNO16, and identified subcutaneous fat pads and fasting plasma triglyceride concentrations as possible contributors to ARC differences observed between DA and COP rats. The lack of significantly increased performance in the COP.DA(chr 3) strain compared with COP rats may stem from their lack of at least one DA rat allele at each of the two epistatic ARC QTLs identified in the $F_2$(COP×DA) genome scan.

## MATERIALS AND METHODS

*Animals.* Inbred DA (DA/OlaHsd) and Copenhagen 2331 (COP/Hsd) rats were purchased from Harlan Sprague-Dawley (Indianapolis, IN) and used to establish a colony housed within the animal care facilities at the University of Toledo College of Medicine. All genetic crosses, congenic, and inbred rats used in the present study were bred from this colony. Rats were weaned at 28 days of age and housed two or three per cage on a 12:12 h light-dark cycle with the light cycle coinciding with daytime. Standard rat chow (Ralston Purina, diet 5001) and water were provided ad libitum. All breeding and experimental procedures were carried out with the approval of the Institutional Animal Care and Use Committee of the University of Toledo, Health Science Campus in accordance with the "Guiding Principles in the Care and Use of Animals" as approved by the Council of the American Physiological Society.

*Construction of congenic strains.* RNO3 and RNO16 intervals containing putative high-capacity ARC QTL alleles from DA rats were separately introgressed into the COP rat genetic background using a marker-assisted breeding (i.e., "speed congenic") approach (27, 38), resulting in the COP.DA(*D3Rat233-D3Mgh14*) congenic and COP.DA(*D16Rat12-D16Rat90*) consomic strains, respectively. Hereafter these congenic strains will be referred to as COP.DA(chr 3) and COP.DA(chr 16). The breeding paradigm was as follows: male $F_1$ rats, bred by crossing male COP rats with female DA rats, were backcrossed to female COP rats. Male progeny heterozygous for the RNO3 and RNO16 ARC QTL-containing regions (loci genotyped are described below for each congenic strain) and containing the fewest number of DA rat alleles at the other loci in the genome were selected for backcrossing to female COP rats. After the first backcross generation, COP.DA(chr 3) and COP.DA(chr 16) congenic strains were developed independently. For each generation, the male rat heterozygous for all markers in the ARC QTL-containing region and carrying the fewest DA rat alleles in the remainder of the genome was bred with up to eight female COP rats. A total of four and five backcross generations were required to breed the COP.DA(chr 3) and COP.DA(chr 16) congenic strains, respectively. Male and female rats heterozygous for the RNO3 or RNO16 ARC QTL-containing regions and lacking DA rat alleles in the background were then mated to fix the DA rat alleles in the congenic regions and COP rat alleles everywhere else. Brother-sister mating was subsequently used to maintain congenic rat strains.

*Genotyping.* DNA was extracted from tail biopsy samples with kits (DNeasy Tissue Kits and DNeasy 96 Tissue Kits; Qiagen, Chatsworth, CA). PCR amplification and gel electrophoresis were performed as previously described (39). Primers used to amplify polymorphic microsatellite markers were purchased from IDT Technologies (Coralville, IA). PCR products were fractionated on 4% agarose gels (Metaphor; Cambrex, Rockland, ME) and visualized with ethidium bromide staining with ultraviolet illumination. PCR products undistinguishable on agarose gels were amplified with $^{32}$P-labeled primers, and the PCR products were resolved on 8% polyacrylamide gels and visualized by autoradiography.

Microsatellite markers for genotyping were chosen from those used in the previous ARC QTL genome scan (39). Initially, five markers, spaced an average of 16.6 cM apart between *D3Rat56* and *D3Rat21*, were used to introgress the RNO3 ARC QTL-containing region. Eight markers, spaced an average of 10.2 cM apart between *D16Rat12* and *D16Rat90,* were used to introgress the DA rat RNO16. A total of 105 markers, spaced an average of 17.5 cM apart, were used to genotype the rats during the selection of the two congenic strains.

Both congenic strains were then genotyped at 84 additional loci that were used in the initial $F_2$(COP×DA) genome scan (39), resulting in a total of 187 loci tested in the two congenic strains as follows. COP.DA(chr 3) was genotyped at 180 loci, spaced 11.2 cM apart on average, to test for the presence of DA rat alleles at locations other than the proximal portion of RNO3. COP.DA(chr 16) was genotyped at 177 loci, spaced 11.4 cM apart on average, to test for the presence of DA rat alleles at chromosomes other than RNO16. All background loci were homozygous for COP rat alleles in the congenic strains.

Additional loci were genotyped to further characterize the introgressed regions of both congenic strains. As these markers were not used in the $F_2$(COP×DA) genome scan (39) distances between loci are expressed in megabases (Mb). Locations of microsatellite markers on RNO3 and RNO16 were obtained from the rat genome sequence database (Build 3.4) at the National Center for Biotechnology Information web site (http://www.ncbi.nlm.nih.gov/genome/guide/rat/index.html). Seventeen additional markers were genotyped in the proximal portion of RNO3, resulting in an average marker spacing of 5.4 Mb in this interval. All of these loci were homozygous for DA rat alleles in the COP.DA(chr 3) congenic strain. Ten additional markers were genotyped on RNO16, resulting in an average marker spacing of 5.0 Mb on this chromosome. All of these loci were homozygous for DA rat alleles in the COP.DA(chr 16) consomic strain.

*ARC phenotype.* A ramped test for maximal treadmill running capacity was used to assess ARC as previously described (1, 20, 39). At 10 wk of age, male COP ($n$ = 36), DA ($n$ = 28), COP.DA(chr 16) ($n$ = 24), and COP.DA(chr 3) ($n$ = 39) underwent an education week to become acclimated to running on the treadmill (model Exer-4; Columbus Instruments, Columbus, OH). At 11 wk of age, rats were tested for maximal treadmill running capacity on five consecutive days. The test consisted of a 10 m/min starting speed that increased 1 m/min every 2 min at a constant 15° grade. Rats were removed from the treadmill at the point of exhaustion, operationally defined as the third time a rat would sustain 3 s on a shock grid (1.2 mAmp at 3 Hz) located at the back of the treadmill lane rather than run. At the end of each run, the rat was taken off the treadmill, and its body weight was measured.

The best distance run to exhaustion from the 5 days of testing was taken as the distance most closely associated with the genetic component of ARC, as previously described (1, 18, 20). The best distance run to exhaustion was also the phenotypic trait used to identify ARC QTLs in the segregating $F_2$(COP×DA) population (39) and has been successfully used to selectively breed high- and low-endurance running capacity rat strains from a genetically heterogeneous stock (18, 20, 40).

Rats tested for endurance running capacity were allowed to recover for 3 wk to avoid possible training effects on other phenotypic measures. These rats were then divided into fasting and ad libitum-fed subsets and used for further experiments to measure body and organ weights (ad libitum-fed animals only), as well as blood chemistries (fasted and ad libitum-fed animals), when they were 15 wk of age (see below).

*Ad libitum-fed organ weight and plasma measurements.* An ad libitum-fed subset of the previously run, 15-wk-old rats [COP ($n$ = 15), DA ($n$ = 12), COP.DA(chr 16) ($n$ = 8), and COP.DA(chr 3) ($n$ = 18)] was used to obtain organ weights and plasma samples. Rats were weighed and then anesthetized with pentobarbital sodium (50 mg/kg body wt). A midline incision was made in each rat from the abdomen through the lower portion of the thoracic cavity. An 18-gauge,

1.5-inch needle (Becton Dickenson, Franklin Lakes, NJ) was inserted into the right ventricle through the apex of the heart, and blood was drawn into $K_2$EDTA-coated Vacutainer tubes (Becton Dickenson, Franklin Lakes, NJ) and set on ice. After blood collection, hearts were removed, blotted dry, and weighed. Left ventricles were isolated by cutting the atria and major blood vessels from both ventricles, along with the top portion of the left ventricle and septum to avoid contamination. The right ventricle was then bisected and cut away proximal to the left ventricle and septum, after which the left ventricle was blotted dry and weighed. The liver was removed as a single unit, blotted dry, and weighed. The pancreas was dissected free from its attachment to the spleen and then medially toward the duodenum, blotted dry, and weighed. Each kidney was separately removed, decapsulated, blotted dry, and weighed.

Abdominal fat content was determined as follows: first, flaps of skin consisting of subcutaneous tissue were freed from abdominal wall tissue from the sternum to the pubic symphysis and displaced laterally. Subcutaneous abdominal fat was dissected free from the skin between the xiphoid process and the rostral border of the pelvic girdle. The abdominal cavity was opened, and the gonadal and mesenteric fat pads were removed and taken together as visceral abdominal fat. Retroperitoneal fat was then dissected free from the underlying musculature and kidneys. The weight of each dissected fat pad was recorded.

*Fasting plasma measurements.* Another subset of the previously run, 15 wk-old rats [COP ($n = 20$), COP.DA(chr 16) ($n = 15$), and COP.DA(chr 3) ($n = 20$)] were fasted by food and water deprivation for 16 h (8–12 PM). Rats were weighed and anesthetized with pentobarbital sodium (50 mg/kg body wt), the thoracic cavity was opened, and whole blood was collected via right ventricular puncture as described above. Blood glucose was measured with an Accu-Check Advantage blood glucose monitor and appropriate test-strips (Roche Diagnostics, Indianapolis, IN).

Following collection, blood samples were centrifuged at 2,800 *g* for 15 min at 4°C. Plasma was then aliquoted and stored at −80°C until assayed. Plasma triglyceride and nonesterified free fatty acid values from each rat were assayed using the same aliquot, on the same day. Plasma insulin was assayed separately using different aliquots of only the highest quality samples to avoid potential interference with the ELISA protocol (see below).

Plasma triglycerides were assayed using a kit [Triglyceride (GPO) Reagent Set, Pointe Scientific; Lincoln Park, MI] with modifications for use with 96-well microtiter plates. Briefly, 3 μl of plasma or diluted standards were added in triplicate to wells, 300 μl of reagent were added to each well, and the plate was covered and incubated at 37°C for 5 min. Absorbance of the colorimetric reaction was measured at 540 nm on a Versamax tunable microplate reader using SoftMax Pro 4.7.1 analysis software (Molecular Devices, Sunnyvale, CA). Plasma nonesterified free fatty acids were assayed using a kit (NEFA C; Wako Chemicals USA, Richmond, VA), also with modifications for use with 96-well microtiter plates. Plasma or diluted standards (5 μl) were added in triplicate to wells and assayed according to manufacturer's specifications at 1/10th scale. Absorbance of the colorimetric reaction was measured at 550 nm with a Versamax tunable microplate reader using SoftMax Pro 4.7.1 analysis software (Molecular Devices). Plasma insulin was assayed with a kit (Rat Insulin ELISA Kit; Crystal Chem, Downers Grove, IL) according to the manufacturer's specifications, including quality of the plasma samples.

*Statistical analysis.* We used SPSS 13.0 for Windows to perform statistical analyses. A Shapiro-Wilk test was used to determine whether data were normally distributed, followed by a Levene test for homogeneity of variance. Normally distributed data were analyzed by a one-way ANOVA to determine the overall level of significance. Data showing overall significance were further analyzed by a Dunnett's post hoc *t*-test to determine interstrain significance, comparing all groups to COP when equality of variance was observed. When homogeneity of variance was not observed (Levene statistic, $P \leq 0.05$), the Dunnett's C post hoc test was used to determine whether interstrain differences were significant.

Data not normally distributed were transformed, and the statistical procedures described above followed. Data still having a nonnormal distribution had extreme outliers removed by the box-plot method on untransformed data followed by analysis using the statistical procedures described above for unaltered data. Data still having a nonnormal distribution were subjected to nonparametric Kruskal-Wallis tests to determine the overall level of significance, followed by a Mann-Whitney pair-wise comparison *U*-test if significant differences were observed. $P < 0.05$ was selected as the criterion for statistical significance.

## RESULTS

A marker-assisted breeding paradigm was used to develop congenic strains where chromosomal intervals on RNO3 and RNO16 containing ARC QTLs from DA rats (39) were introgressed onto an otherwise uniform background of COP alleles, resulting in the COP.DA(chr 3) and COP.DA(chr 16) congenic strains, respectively. The congenic region of COP.DA(chr 3) was a 118- to 135.2-Mb interval of RNO3 with the region known to contain DA rat alleles extending from *D3Rat233* to *D3Mgh14*. The congenic region of COP.DA(chr 16) was an 85.3- to 90.2-Mb interval of RNO16, with the region containing DA rat alleles extending from *D16Rat12* to *D16Rat90*. The congenic region of COP.DA(chr 16) encompasses 95–100% of RNO16 and should thus be considered a consomic strain. Physical maps of the introgressed chromosomal regions of the COP.DA(chr 16) consomic strain and COP.DA(chr 3) congenic strains are shown in Fig. 1.

In the present study, we observed a 412.7-m (41.9%) greater best distance run to exhaustion for DA rats (984.6 ± 38.5 m) compared with COP rats (571.9 ± 27.5 m, Fig. 2), consistent with our previous results (1, 19, 23, 39). COP.DA(chr 16) rats had a significantly greater mean best distance run to exhaustion of 696.7 ± 38.2 m compared with COP rats ($P = 0.03$; Fig. 2). COP.DA(chr 3) rats, however, had a mean best distance run to exhaustion of 643.6 ± 40.9 m, and while greater than that of COP rats, it was not statistically significant ($P = 0.21$).

No significant correlation was observed between strain and the day when the best distance run to exhaustion occurred over the 5-day period of the trial ($r = 0.084$, $P = 0.35$). No significant strain differences were observed for the mean of the day of occurrence of the best distance run to exhaustion ($P = 0.94$), *day 2.56* for COP.DA(chr 3), *day 2.63* for COP.DA(chr 16), *day 2.81* for COP, and *day 3.30* for DA rats.

DA and COP.DA(chr 16) rats both had significantly longer average distance run to exhaustion over the 5 days of testing (755.6 ± 35.0 m, $P < 0.001$ and 517.5 ± 27.6 m, $P = 0.012$, respectively) compared with COP rats (406.2 ± 17.2 m). While COP.DA(chr 3) rats ran for a greater distance (463.7 ± 30.6 m) compared with COP rats, this difference was not statistically significant ($P = 0.14$).

Significant differences in organ weights were observed between COP and DA rats for all organs measured, excepting the pancreas and kidneys. No significant differences in heart or left ventricular weights were observed between either congenic strain compared with the parental, COP strain (Table 1). COP.DA(chr 16) and COP rats did not show significant differences in body weight (Table 1). However, COP.DA(chr 3) rats had a significantly lower body weight compared with COP

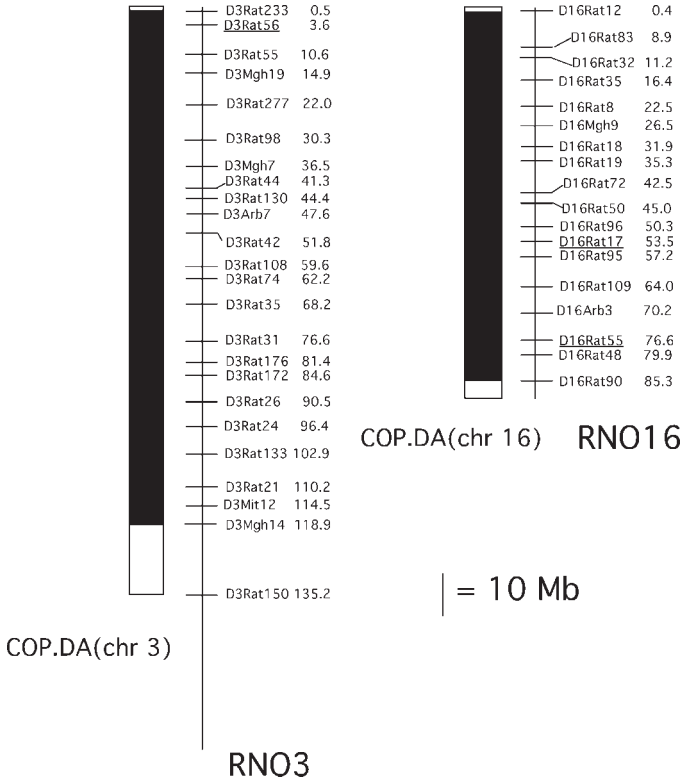


Fig. 1. Physical maps showing the extent of the congenic regions carried by the COP.DA(chr 3) and COP.DA(chr 16) strains. Putative quantitative trait locus (QTL)-containing regions on RNO3 and RNO16 were transferred by successive rounds of marker assisted backcrossing from the DA (donor) strain into the Copenhagen (COP, recipient) strain. Solid bars depict introgressed regions in the congenic and consomic strains that contain DA rat alleles; open bars indicate chromosomal regions where crossovers have occurred (and which contain the boundaries of the congenic region), and solid lines represent the length of the entire chromosome. Loci located at or near aerobic running capacity (ARC) QTL peaks, as described in Ways et al. (39), are underlined. The locations of microsatellite markers on RNO3 and RNO16 were obtained from the rat genome sequence database (Build 3.4) at the National Center for Biotechnology Information web site (http://www.ncbi.nlm.nih.gov/genome/guide/rat/index.html), with map distances expressed in megabases (Mb).

rats (252.6 ± 4.6 g vs. 271.0 ± 5.5 g, $P = 0.014$; Table 1) and were comparable to that of DA rats (257.0 ± 3.5 g; Table 1). Body weight-adjusted heart and left ventricular weights were significantly higher for COP.DA(chr 3) compared with COP rats (2.99 ± 0.06 mg/g vs. 2.76 ± 0.03 mg/g and 1.96 ± 0.05 mg/g vs. 1.75 ± 0.02 mg/g, respectively; Table 1). COP.DA(chr 3) rats had significantly lower pancreas weights compared with COP rats (0.52 ± 0.02 g vs. 0.62 ± 0.03 g, $P < 0.05$), although body weight-adjusted pancreas weights were not significantly different (Table 1). In addition, COP.DA(chr 3) rats had a significantly lower kidney weight compared with COP rats (1.61 ± 0.05 g vs. 1.84 ± 0.05 g, $P = 0.002$; Table 1). No significant differences in liver weight were observed between COP.DA(chr 16) and COP.DA(chr 3) congenic rats compared with COP rats (Table 1).

DA rats had significantly more total abdominal fat compared with COP rats (7.93 ± 0.34 g vs. 4.95 ± 0.22 g, $P < 0.001$; Fig. 3). Separating total abdominal fat into its retroperitoneal, subcutaneous, and visceral components revealed that DA rats had significantly more of each component compared with COP rats (Fig. 3). COP.DA(chr 16) consomic rats also had significantly more total abdominal fat (6.47 ± 0.24 g vs. 4.95 ± 0.20 g, $P = 0.001$; Fig. 3) and subcutaneous abdominal fat (2.25 ± 0.09 g vs. 1.27 ± 0.11 g, $P < 0.05$; Fig. 3) compared with parental COP rats, with the majority of the difference in total abdominal fat in COP.DA(chr 16) rats stemming from the large difference in subcutaneous abdominal fat (23% higher compared with COP rats).

Significant differences between either congenic strain compared with COP rats were not observed with regard to fed or fasted plasma triglycerides, nonesterified free fatty acids, or glucose (fasted only) levels, with the exception of lower fasted plasma triglycerides levels observed in COP.DA(chr 16) rats ($P < 0.05$, Table 2). No significant strain differences in fasted plasma insulin levels were observed between both COP.DA(chr 3) and COP.DA(chr 16) rats compared with COP rats (Table 2).

## DISCUSSION

The greater mean best distance run to exhaustion observed for the COP.DA(chr 16) consomic strain compared with the parental COP strain indicates that introgression of DA rat RNO16 alleles into the COP rat improves ARC, confirming the influence from an ARC QTL on RNO16. Similarly, the failure to observe a significantly greater treadmill endurance running capacity for COP.DA(chr 3) rats is consistent with the results of our previous ARC genome scan in a segregating $F_2$(COP×DA) population (39). In the aforementioned genome scan we observed an interaction between loci on RNO16 (*D16Rat55*) and RNO3 (*D3Rat56*) such that a minimum of one DA rat allele was needed at each locus to observe a greater distance run to exhaustion on the treadmill (39). COP.DA(chr 16) and COP.DA(chr 3) congenic strains are homozygous for DA rat alleles at *D16Rat55* and *D3Rat56*, respectively, but homozygous for COP rat alleles at the other loci (*D3Rat56* and *D16Rat55*, respectively). Thus, positive epistatic interactions

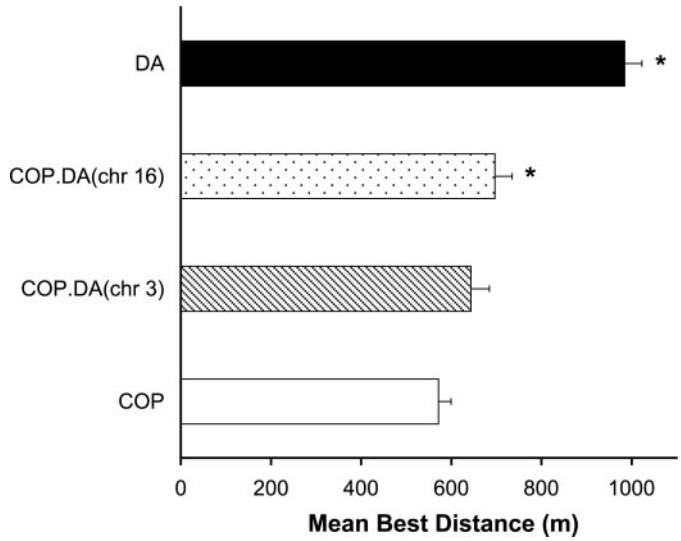


Fig. 2. Mean best distance run for congenic strains and parental COP and DA rats. Distances run to exhaustion are as follows: DA = 985 ± 39 m ($n = 28$), COP.DA(chr 16) = 697 ± 38 m ($n = 24$), COP.DA(chr 3) = 644 ± 41 m ($n = 39$), COP = 572 ± 28 m ($n = 36$). Values are given as means ± SE. DA rats showed significantly greater performance compared with each of the other strains ($P < 0.0001$). *Significantly different from the COP rat value ($P < 0.05$).

Table 1. *Anatomical comparisons of congenic and inbred strains*

| Variable | COP | COP.DA(chr 16) | COP.DA(chr 3) | DA |
|---|---|---|---|---|
| *n* | 15 | 8 | 18 | 12 |
| BW, g | 271.0±5.5 | 275.6±4.6 | 252.6±4.6* | 257.0±3.5* |
| Heart weight, g | 0.76±0.01 | 0.73±0.02 | 0.75±0.02 | 0.85±0.02* |
| BW-adjusted heart weight, mg/g† | 2.76±0.03 | 2.65±0.04 | 2.99±0.06* | 3.29±0.05* |
| Left ventricle weight, g | 0.49±0.01 | 0.48±0.01 | 0.49±0.02 | 0.55±0.01* |
| BW-adjusted left ventricle weight, mg/g† | 1.75±0.02 | 1.75±0.02 | 1.96±0.05* | 2.13±0.04* |
| Pancreas weight, g | 0.62±0.03 | 0.49±0.04 | 0.52±0.02* | 0.53±0.04 |
| BW-adjusted pancreas weight, mg/g | 2.29±0.12 | 1.79±0.12 | 2.05±0.09 | 2.09±0.19 |
| Liver weight, g | 9.55±0.16 | 9.40±0.16 | 9.24±0.31 | 8.02±0.19* |
| BW-adjusted liver weight, mg/g‡ | 35.3±0.45 | 34.2±0.67 | 36.5±0.88 | 31.2±0.51* |
| Total kidney weight, g | 1.84±0.05 | 1.79±0.05 | 1.61±0.05* | 1.99±0.05 |
| BW-adjusted total kidney weight, mg/g | 6.80±0.14 | 6.49±0.18 | 6.37±0.11* | 7.73±0.13 |

Values are means ± SE. Body weight (BW)-adjusted values represent organ weights that were normalized to body weight. †Variables with extreme outliers removed from Copenhagen (COP) rats ($n = 14$). ‡Adjusted liver weight transformed using 1/(liver weight/body weight). *Significantly different from the COP rat value ($P < 0.05$).

between these two QTLs would not be expected to occur in either the COP.DA(chr 16) consomic or COP.DA(chr 3) congenic strains. The greater endurance running performance observed in COP.DA(chr 16) consomic rats compared with COP rats is likely a product of the significant ARC QTL identified near *D16Rat17* in the genome scan (39).

Previously, our laboratory identified a number of physiological differences that could contribute to the strain differences in aerobic performance observed between inbred COP and DA rats. Barbato et al. (1) reported a significantly greater cardiac output in DA rats compared with COP rats as measured by the Langendorff-Neely isolated working heart preparation. Other cardiovascular variables for which DA rats showed improved function over COP rats include sympathetic and parasympathetic support of blood pressure at rest, rates of tension change in isolated papillary muscles, and the amplitude of the calcium transient during contraction leading to an increased fractional shortening (8, 19). However, these physiological strain differences have not been linked to any specific chromosomal region, making it difficult to identify a contributing genetic factor. In addition, a number of RNA sequences were found differentially expressed between the left ventricles of COP and DA rats, indicating differences in cardiac gene regulation (23). Two of these differentially expressed genes include phosphatidylinositol 3-kinase, regulatory subunit 1 (*Pik3r1*) and the rat ortholog of human myosin light chain 2a gene (*MLC2a*), which both showed >14-fold differences in RNA expression levels but did not map near ARC QTLs. We also identified two differentially expressed genes, mapping near the two RNO16 ARC QTL peaks (39): PDZ and LIM domain 3 (actinin alpha 2-associated LIM protein; *Pdlim3*), which is involved in actin filament organization and muscle development, and TM2 domain-containing 2 (*Tm2d2*), which is of unknown function.

Our laboratory has consistently found greater heart and left ventricular weights for the high-performing DA strain compared with the low-performing COP strain (1, 19, 23, 39). Expression profiling of the left ventricles of these strains also identified two networks of coordinately regulated, differentially expressed genes whose annotations suggested involvement in cell growth, metabolism, death, and differentiation (23). Findings of higher isolated cardiac function for DA, compared with COP rats (1), as well as divergence for a number of related phenotypes (8, 19) described above, further implicate functional cardiac differences as likely contributors to ARC. While heart weight (but not left ventricle

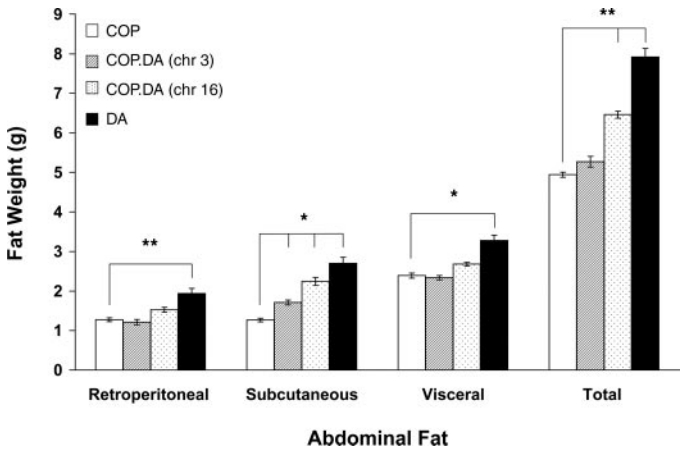


Fig. 3. Abdominal fat content for congenic strains and the parental COP and DA strains. Abdominal fat, as its retroperitoneal, subcutaneous, and visceral fat pad components, was dissected from COP ($n = 15$), DA ($n = 12$), COP.DA(chr 16) ($n = 8$), and COP.DA(chr 3) ($n = 18$) and weighed. Values are given as absolute means ± SE. Total abdominal fat is the sum of the retroperitoneal, subcutaneous, and visceral fat pad components. *Significantly different from COP rat values ($P < 0.05$). **Significantly different from COP rat values ($P \leq 0.001$).

Table 2. *Circulating metabolic substrate levels for COP and congenic rats*

| Plasma Value | COP | COP.DA (chr 16) | COP.DA (chr 3) |
|---|---|---|---|
| Fed | | | |
| *n* | 14 | 8 | 16 |
| Triglycerides, mg/dl | 51.71±8.3 | 39.55±5.02 | 47.59±6.17 |
| Free fatty acids, mEq/l | 0.12±0.01 | 0.13±0.02 | 0.10±0.01 |
| Fasted | | | |
| *n* | 20 | 15 | 20 |
| Triglycerides, mg/dl | 26.03±1.9 | 20.63±2.8* | 30.5±2.5 |
| Free fatty acids, mEq/l | 0.23±0.02 | 0.29±0.03 | 0.22±0.02 |
| Glucose, mg/dl | 119.2±4.34 | 118.1±7.58 | 132.1±5.20 |
| Insulin, ng/ml† | 1.12±0.22 | 1.25±0.29 | 1.02±0.31 |

Values are means ± SE. Plasma glucose and insulin (†10 rats/strain) were only measured in fasted animals. Plasma triglyceride (fed and fasted) and insulin (fasted) concentration values were log transformed to achieve normal distributions. *Significantly different from COP rat values ($P < 0.05$).

weight) and body weight QTLs were observed in the original $F_2$(COP×DA) genome scan (39), they did not cosegregate with ARC. Our finding of a significantly higher ARC in COP.DA(chr 16) rats compared with the parental, COP strain, despite no significant difference in heart or left ventricular weights, confirms that this RNO16 ARC QTL is independent of cardiac mass, though probably not of other functional cardiac phenotypes.

Although subcutaneous fat depots are a major source of energy stores, high aerobic fitness is often associated with lower abdominal fat content, including subcutaneous fat (14, 17, 33, 34, 40, 41). The significantly larger subcutaneous abdominal fat and lower fasting plasma triglyceride levels observed in the COP.DA(chr 16) consomic strain suggest an increased ability to store dietary fat compared with COP rats. Thus, the increased subcutaneous abdominal fat store may not be detrimental but may serve as a store of additional energy and provide a metabolic advantage during endurance exercise, particularly during the low-intensity portion (6, 7).

COP.DA(chr 3) congenic rats had significantly decreased body, pancreas, and kidney weights and significantly increased body weight-adjusted heart and body weight-adjusted left ventricle weights compared with the parental, COP strain, despite the lack of a significantly greater ARC. Some of the above strain differences may have resulted from interactions of introgressed DA rat RNO3 alleles with background COP rat alleles and be unrelated to ARC.

Introgressing both DA rat RNO16 and RNO3 QTL-containing regions into the same COP genetic background would allow confirmation of the epistatic interaction observed in the ARC genome scan, as has been done for other phenotypes in rats (e.g., 29, 31, 37). Such double congenic strains would also facilitate examination of the summed effects of the DA rat alleles that were introgressed into the COP.DA(chr 16) and COP.DA(chr 3) strains, for ARC and the other measured phenotypes.

Studies in both mice and humans have linked maximal exercise capacity to chromosomal regions orthologous to those containing the RNO16 ARC QTLs (Ref. 39 and the present study). Preliminary results from Lightfoot and coworkers (26) identified an aerobic capacity QTL in female mice at *D8Mit359*, orthologous to an interval near the more proximal RNO16 ARC QTL (Fig. 1). A genome scan for endurance performance using a cycle ergometer identified a maximal power output QTL at *D13S796*, orthologous to an interval near the more distal RNO16 ARC QTL at *D16Rat90* (32). While neither QTL mapped within the 1-LOD support interval of an RNO16 ARC QTL (39), the imprecision of QTL localization by genome scans (11) does not preclude similar gene(s) from underlying the two RNO16 aerobic capacity QTLs in mice and rats or humans and rats.

QTLs for several other quantitative traits possibly related to ARC have also been mapped to mouse and human genomic intervals orthologous to RNO16 and the proximal portion of RNO3. Such QTLs mapping within 1-LOD support intervals of the RNO3 and RNO16 ARC QTLs (39) include *1*) mouse chromosome 2 QTLs for lean body mass (28) and type II diabetes mellitus (determined by glucose and insulin concentrations as well as subcutaneous and mesenteric fat weight) and *2*) a mouse chromosome 8 fasting plasma triglyceride QTL (36) orthologous to intervals containing the RNO3 and the more proximal RNO16 ARC QTLs, respectively. Furthermore, the β3-adrenergic receptor (*Adrb3*) and lipoprotein lipase (*Lpl*) genes, both located near RNO16 ARC QTLs, have been associated with differences in fat mass in humans (12, 35). These comparative QTL data are supportive of our findings of differences *1*) in fasting triglyceride concentrations in COP.DA(chr 16) consomic rats compared with COP rats and *2*) in fat mass in both COP.DA(chr 3) and COP.DA(chr 16) rats compared with COP rats.

The COP.DA(chr 16) consomic strain confirmed at least one ARC QTL responsible, in part, for the observed strain differences in ARC between COP and DA rats. The significantly greater amount of subcutaneous abdominal fat and lower plasma triglyceride concentrations in COP.DA(chr 16), compared with COP rats, suggest that genes regulating energy balance and ARC are present in the same RNO16 consomic interval. At present, we cannot distinguish whether these phenotypic differences stem from the same alleles that affect ARC or whether these differences represent distinct QTLs [and gene(s)] present on RNO16. Substitution mapping analysis of RNO16 using congenic substrains carrying smaller regions of introgressed DA rat alleles can be used to determine whether or not ARC and the energy balance phenotypes are indeed linked.

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