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Exercise training alters effect of high-fat feeding on the ACTH stress response in pigs

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Abstract

Eating and physical activity behaviors influence neuroendocrine output. The purpose of this study was to test, in an animal model of diet-induced cardiovascular disease, the effects of high-fat feeding and exercise training on hypothalamo–pituitary–adrenocortical (HPA) axis activity. We hypothesized that a high-fat diet would increase circulating free fatty acids (FFAs) and decrease the adrenocorticotrophic hormone (ACTH) and cortisol response to an acute stressor. We also hypothesized that exercise training would reverse the high-fat diet-induced changes in FFAs and thereby restore the ACTH and cortisol response. Pigs were placed in 1 of 4 groups (normal diet, sedentary; normal diet, exercise training; high-fat diet, sedentary; high-fat diet, exercise training; $n = 8/\text{group}$). Animals were placed on their respective dietary and activity treatments for 16–20 weeks. After completion of the treatments animals were anesthetized and underwent surgical intubation. Blood samples were collected after surgery and the ACTH and cortisol response to surgery was determined and the circulating concentrations of FFAs, glucose, cholesterol, insulin, and IGF-1 were measured. Consistent with our hypothesis, high-fat feeding increased FFAs by 200% and decreased the ACTH stress response by 40%. In exercise-trained animals, the high-fat diet also increased FFA; however, the increase in FFA in exercise-trained pigs was accompanied by a 60% increase in the ACTH response. The divergent effect of high-fat feeding on ACTH response was not expected, as exercise training alone had no effect on the ACTH response. Results demonstrate a significant interaction between diet and exercise and their effect on the ACTH response. The divergent effects of high-fat diet could not be explained by changes in weight gain, blood glucose, insulin, or IGF-1, as these were altered by high-fat feeding, but unaffected by exercise training. Thus, the increase in FFA with high-fat feeding may explain the blunted ACTH response to an acute stressor in sedentary animals, but cannot explain the exaggerated response in exercise trained animals.

Keywords

HPA axis; stress; ACTH; fat diet; cortisol; free fatty acids

Introduction

Physical inactivity, high cholesterol, and diabetes are major risk factors for the development of cardiovascular disease (Pearson et al. 2002). The American Heart Association also lists an individual's stress response as a possible contributor to cardiovascular disease. In this study,

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we attempt to discriminate between the individual and interactive effects of diet and exercise on endocrine and metabolic parameters that occur during cardiovascular disease development.

Altering the activity of the hypothalamo–pituitary–adrenocortical (HPA) axis can contribute to cardiovascular and metabolic disease through changes in circulating glucocorticoid concentrations (Chrousos 2000). The activation of the HPA axis occurs in response to disruptions in homeostasis, or stressors, and results in an increase in circulating glucocorticoids (Miller and O’Callaghan 2002). Activation of the HPA axis occurs in response to both real and perceived threats. Neurons within the paraventricular nucleus integrate information from various sources when determining the magnitude of the HPA stress response (Herman et al. 2002).

Acute exercise is a stressor that can increase the activity of the HPA axis in an intensity-dependent manner (Davies and Few 1973; Luger et al. 1987). Acute exercise does not always induce an HPA response and a decrease in cortisol can be seen during low-intensity (<50% $\text{VO}_{2\text{ max}}$) exercise (Davies and Few 1973). In response to exercise training, the HPA response to the same absolute workload is decreased (Buono et al. 1987). Interestingly, in rodents, exercise training can alter the HPA response to non-exercise stressors (Droste et al. 2003; Fediuc et al. 2006).

Food intake is also a stressor that can increase the activity of the HPA axis and the HPA response to food intake is dependent on macronutrient composition (Vicennati et al. 2002). In rodents, a diet of high-fat meals typically increases both basal and stress-induced activity of the HPA axis (Legendre and Harris 2006; Tannenbaum et al. 1997). Although a high-fat diet may induce multiple effects, one mechanism by which high-fat feeding may affect HPA activity is through circulating free fatty acids (FFAs) (Lanfranco et al. 2004). It has been shown that infusion of a lipid–heparin emulsion increases FFAs and inhibits adrenocorticotrophic hormone (ACTH) and cortisol secretion in humans (Lanfranco et al. 2004). If given during exercise, the lipid–heparin emulsion inhibited the ACTH and cortisol response to exercise in adult males (Coiro et al. 2007).

In a swine model of early stage cardiovascular disease (Thompson et al. 2004; Turk et al. 2003; Turk et al. 2005), we discovered that high-fat feeding decreased the ACTH stress response. We hypothesized that the decreased ACTH response in the high-fat diet group was the result of increased circulating FFAs and that exercise training would reverse the high-fat diet-induced decrease in ACTH response by decreasing circulating FFAs. Previous work in this pig model demonstrated that the high-fat diet stimulates development of atherosclerotic plaques and vascular lesions (Turk et al. 2003; Turk and Laughlin 2004). To further characterize this disease-relevant model and to examine other factors that may contribute to divergent ACTH responses, we have also examined the effects of diet and exercise training on clinical chemistry profiles and plasma insulin and IGF-1 concentrations.

Materials and methods

Animals

Male Yucatan miniature swine (Sinclair Research Farm, Columbia, Mo.) were used for this study and all experiments were approved by the University of Missouri institutional animal care and use committee. Pigs were around 6 months of age upon arrival to our animal facility and between 11 and 13 months of age at the time of blood and tissue collection. Pigs were placed in 1 of 4 different groups (normal diet, sedentary; normal diet, exercise training; high-fat diet, sedentary; high-fat diet, exercise training). Animals were placed on their respective dietary and activity treatments for 16–20 weeks.

Prior to initiation of treatments blood samples were collected and animals completed a maximal exercise test to determine endurance capacity. The maximal exercise test involved incremental increases in treadmill speed and grade until the pig was no longer able to keep pace with the treadmill (i.e., time to exhaustion). Such a test was also completed at the end of treatments. Body weights, blood samples, and heart weights were obtained post-treatment. This study was first completed in a set of 20 animals ($n = 5/\text{group}$) and the results confirmed in a second set of 12 animals ($n = 3/\text{group}$). Results represent the combination of data from the 2 sets of experimental animals ($n = 8/\text{group}$).

Diet and exercise regimes

The pigs on the normal diet received standard mini-pig chow (Laboratory Mini-Pig Breeder diet, Constant Nutrition) with macronutrient composition as follows: carbohydrate, 69%; protein, 23%; and fat, 8%. Animals on the high-fat diet had standard mini-pig chow with coconut oil (17% total volume), corn oil (2%), cholesterol (2%), and sodium cholate (0.7%) added resulting in the following macro-nutrient composition: carbohydrate, 41%; protein, 13%; and fat, 46%. Animals were fed equalcaloric meals once daily.

Pigs undergoing exercise training completed treadmill running 5 d/week for about 1.5 h throughout the exercise-training period. Duration and treadmill speed were increased throughout the training period and by the end of the study animals were running approximately 8 km/d (5 min warm-up, ~25% $\text{VO}_{2\text{ max}}$; 15 min at 10–12 km/h, ~90% $\text{VO}_{2\text{ max}}$; 60 min at 7–8 km/h, ~75% $\text{VO}_{2\text{ max}}$; and 5 min cool-down, 25% $\text{VO}_{2\text{ max}}$). Exercise was completed the day before the collection of post-treatment blood samples.

Stressors and blood collection

The HPA response to an acute stressor was evaluated by measuring ACTH and cortisol after exposure to anesthesia and surgery. Following an overnight fast, between 0800 and 1000 h, animals were sedated with ketamine (35 mg/kg, i.m.) and xylazine (2.25 mg/kg, i.m.), and anesthetized with thiopental (35 mg/kg). Approximately 30 min after being sedated animals underwent surgical intubation of the trachea (~5 min procedure) and cannulation of the external jugular vein. Approximately 10 min after the initiation of surgery blood samples were collected in vacutainers for plasma (EDTA) and serum, spun in a centrifuge, aliquoted, and stored at -80°C until analysis.

To determine the basal HPA rhythm in Yucatan pigs we collected automated blood samples (Accusampler, DiLab) in a separate group of pigs on the normal diet. This group of animals underwent surgery to have vascular access ports connected to the jugular vein. After at least 1 week of recovery animals were connected to blood collection equipment and samples were withdrawn every hour for a 24 h period. This allowed blood to be collected without disturbing the animals; it is also important to note that connecting animals to blood collection equipment did not limit their ability to move around in their pen.

Blood glucose (Infinity) and non-esterified fatty acids (NEFA; Wako) were measured by absorbance assays. ACTH and cortisol were measured by chemiluminescent assays (Immulite, DPC). Serum was delivered to University of Missouri Veterinary Medical Diagnostic Laboratory for clinical chemistry profiles to examine the possible influence of diet and exercise on other physiological systems. Insulin and IGF-1 were measured by radioimmunoassay at the Diagnostic Center for Population and Animal Health (Michigan State University).

Statistical analyses

Two-way analysis of variance (ANOVA) tests with Holm–Sidak pairwise multiple-comparison method were used to determine the effects of diet and activity. One-way ANOVA

tests were used to compare clinical chemistry variables collected prior to initiation of diet and activity treatments. Data presented as mean $\pm$ SE, $p < 0.05$.

Results

Animals

Table 1 presents the clinical chemistry profiles of the 4 different study groups at the beginning of our study. Creatinine was the only marker that had a statistically significant difference between groups.

Body weight and exercise capacity

There was a main effect of diet on body weight and heart weight, but not on heart – body weight ratio (Table 2). There was also a main effect of exercise training on heart weight and heart – body weight ratio, but not on body weight. There were no statistically significant interactions between diet and exercise with body and heart weights or exercise capacity. There was a main effect of exercise training on exercise time to exhaustion, providing evidence for the efficacy of the exercise-training protocol to increase endurance capacity. The high-fat diet had a negative main effect on exercise time to exhaustion.

HPA axis activity

Figure 1 shows 24 h hormonal rhythms for ACTH (Fig. 1A) and cortisol (Fig. 1C) in undisturbed, normal-diet pigs. The ACTH (Fig. 1B) and cortisol (Fig. 1D) response to the anesthesia and surgery is shown for normal diet and high-fat diet sedentary and exercise-trained pigs. Mean basal daytime concentrations of ACTH (15.4 ± 0.7) and cortisol (47.7 ± 2.8) were determined using automated blood sampling equipment. In comparison to resting daytime values, there was a stress-induced increase in ACTH and cortisol of approximately 10 and 2.5 fold, respectively, in normal diet pigs. There was a significant interaction between diet and exercise on the ACTH stress response ($p = 0.02$). In sedentary animals the ACTH stress response was blunted with the high-fat diet. In animals on the high-fat diet, exercise training significantly increased the ACTH stress response. There were no differences in plasma cortisol at time of blood collection.

Cholesterol and FFA

There was a main effect of diet on serum cholesterol (Fig. 2A) and FFAs (Fig. 2B). There was a main effect of exercise on FFAs, but not on serum cholesterol. In sedentary animals, the high-fat diet increased FFAs by 228% in comparison with normal-diet animals. Relative to sedentary animals, exercise training decreased FFAs by 45% and 56% in animals on the normal and high-fat diets, respectively. There was a positive correlation between FFAs and stress-induced ACTH in exercise-trained pigs on the normal diet ($p = 0.03$, data not shown). There was not a statistically significant correlation between FFAs and ACTH within the other groups ($p > 0.75$). Also, there was not a statistically significant correlation between FFAs and stress-induced ACTH when all animals were included in the analysis ($p = 0.6$) or within activity (exercise, $p = 0.2$; sedentary, $p = 0.5$), or dietary (normal diet, $p = 0.97$; high-fat diet, $p = 0.5$) treatments.

Clinical chemistry profiles

There was a main effect of diet on serum alanine transaminase, albumin, BUN, alkaline phosphatase, and phosphate (Table 3). There was a main effect of exercise training on alanine transaminase, alkaline phosphatase, and phosphate. There were no statistically significant interactions between diet and exercise. There was a main effect of diet on serum glucose, insulin, and IGF-1 (Fig. 3). There was not a main effect of exercise on serum glucose, insulin, or IGF-1.

Discussion

In this study, we report some of the metabolic and endocrine changes that occur during the development of an early stage of cardiovascular disease (Thompson et al. 2004). The high-fat diet reduced the ACTH stress response in sedentary animals but, interestingly, resulted in an exaggerated ACTH response in exercise-trained animals. Importantly, these results were confirmed in two sets of experimental animals. The current data also reveal that high-fat feeding resulted in statistically significant changes in cholesterol, FFAs, glucose, albumin, phosphate, alanine transaminase, alkaline phosphatase, BUN, insulin, and IGF-1. Of all factors altered by high-fat feeding, only FFAs and the ACTH stress response were seen to interact with effects of exercise training.

One goal of this study was to test the hypothesis that differences observed in the ACTH stress response would occur alongside differences in blood FFA concentrations. Consistent with this hypothesis we found that sedentary animals on the high-fat diet had increased FFAs and a decreased ACTH response to an acute stressor. These observations in sedentary animals are in agreement with previous evidence in humans demonstrating that increased blood FFAs decreases HPA activity (Lanfranco et al. 2004).

The high-fat diet increased FFAs in both sedentary and exercise trained animals. However, the ACTH stress response, which was blunted in the sedentary high-fat pigs, was enhanced in the high-fat diet, exercise-trained group. Thus, the altered ACTH response in exercise-trained animals on the high-fat diet cannot be explained by the same mechanism suggested in the sedentary animals (i.e., increased FFAs cause decreased HPA response). Furthermore, other factors by which exercise training could have altered the ACTH response, such as body weight gain, blood glucose, insulin, or IGF-1, were unaffected by exercise training and therefore do not explain the divergent effects of the high-fat diet on sedentary and exercise-trained groups. It is possible that additional stressors involved with exercise training, e.g., moving the animal from pen to treadmill, contributed to the effect of exercise on the HPA axis response.

Despite group differences in the ACTH response no difference in the cortisol response was observed. Several possible explanations for the lack of difference in cortisol despite differences in ACTH include (i) a delay between the increase in ACTH and cortisol and blood collection at a single timepoint that allowed observation of ACTH differences, but prevented observation of cortisol differences that may have occurred at a later time point; (ii) a non-linear relationship between ACTH and cortisol; (iii) a decrease in adrenal sensitivity with anesthesia preventing cortisol synthesis from reflecting ACTH differences; (iv) adrenal sensitivity to ACTH was changed with diet or exercise; and (v) differences in stress-induced ACTH levels are simply not always reflected in differences in stress-induced corticosteroids (Ostrander et al. 2006). Importantly, it is possible for changes in ACTH to affect vascular function even without changes in cortisol, as ACTH can have direct effects on endothelial cells, which express ACTH receptors (Hatakeyama et al. 2000).

It is possible that the inhibitory effect of FFAs was removed in the exercise-trained animals and (or) that there is a net increased excitatory drive to the paraventricular nucleus when animals receive exercise training and high-fat feeding concurrently. It is also possible that the contribution of redundant neuroendocrine pathways and (or) peptides capable of eliciting ACTH release increase in response to exercise training and that these pathways or peptides may be insensitive to FFA inhibition. Indeed, the fact that we observed a statistically significant positive correlation between FFAs and the ACTH response in exercise-trained animals on the normal diet suggests that the relationship between FFAs and neuroendocrine activity of the HPA axis had been altered. The altered relationship between FFAs and the ACTH response in exercise-trained animals may explain why exercise-trained animals on the high-fat diet

demonstrated the most robust ACTH response of all 4 groups, whereas sedentary animals on the high-fat diet had a blunted response.

During acute exercise an HPA response is induced and there is an increase in FFAs (Goto et al. 2007). Perhaps repeated bouts of an acute stressor that requires HPA activation at a time when FFAs are increasing leads to a loss of sensitization of the HPA axis to their inhibitory effects. In rats, previous reports have shown an increased stress response with high-fat feeding (Legendre and Harris 2006; Pascoe et al. 1991; Tannenbaum et al. 1997), although that is not always the case (La Fleur et al. 2003; Pecoraro et al. 2006). Perhaps metabolic rate and (or) fitness level of the animal determines the influence that high-fat feeding and circulating FFAs have on the HPA response to an acute stressor. Indeed, high-fat feeding exaggerates the stress response in rats and, as shown in this study, in exercise-trained pigs, whereas high-fat feeding in humans and sedentary pigs leads to an inhibition of HPA activity.

Based on our experience, we expected this high-fat diet to produce a large increase in total, high-density lipoprotein, and low-density lipoprotein cholesterol, as well as increases in blood triglycerides (Thomas et al. 2002). Previous work also found no effect of exercise training and no evidence of exercise–diet interactions on blood triglycerides, cholesterol, or lipoproteins (Thomas et al. 2002). Because exercise training did not alter cholesterol levels in our pigs, our study confirms these previous results and is consistent with many (Durstine et al. 2001; Jacobs et al. 2006), but not all (Halbert et al. 1999), previous reports in human subjects.

The increases in glucose and FFAs observed in the high-fat diet pigs suggest that metabolism was perturbed by this diet. In further support of this we also observed statistically significant increases in serum insulin and IGF-1. Previous evidence has shown that 2 weeks of high-fat feeding in rats resulted in significant increases in FFAs and insulin and that both indices could be improved by treatment with an A₁ adenosine receptor agonist (Dhalla et al. 2007). Exercise training decreased FFA levels in our pigs, but did not affect insulin concentrations. Evidence from overweight humans has suggested that exercise training may improve insulin sensitivity through its ability to decrease FFAs (Shojaee-Moradie et al. 2007). Perhaps the reason that we did not see a beneficial effect of exercise training on insulin concentrations in high-fat fed animals was that the magnitude of change in FFAs produced by the diet was too great (increased by 228%). As a result, even though exercise training attenuated this increase by over 50%, the FFA concentration in the exercise-trained, high-fat diet pigs was still twice that of the normal-diet animals.

Our results suggest that the high-fat diet produced alterations in liver and renal function reflected by the statistically significant differences in clinical chemistry profiles. Of markers affected by high-fat feeding, serum albumin (Djousse et al. 2002; Gillum and Makuc 1992; Schalk et al. 2006; Weijenberg et al. 1997), alkaline phosphatase (Hanley et al. 2005), alanine transaminase (Hanley et al. 2005; Schindhelm et al. 2006; West et al. 2006), BUN (Kirtane et al. 2005; Pu et al. 2006), and phosphate (Ishimura et al. 2005; Tonelli et al. 2005) have all been associated with metabolic and (or) cardiovascular disease. Our observation of a decrease in serum albumin in response to high-fat feeding confirms previous results in rats (Ghasi et al. 2000). It is interesting that exercise training did not reverse any of these high-fat diet-induced changes. It is important to emphasize that the decreases in albumin, alanine transaminase, and BUN seen in the high-fat diet pigs may be epiphenomena produced by reducing protein in the diet that was displaced by increased fat. Indeed, although markers of liver and renal function were statistically different, these markers remain compatible with normal hepatic and renal function.

It is not surprising that evidence provided in our study demonstrates that diet and exercise are unique in the manner by which they affect physiological function. Indeed, many of the changes

induced by high-fat feeding were unaffected by exercise training. These results suggest that although exercise, or increased levels of physical activity, can increase energy expenditure to negate increased caloric intake, exercise should not be viewed as a cure for poor dietary habits. Exercise training affected only a portion of the multi-factorial effects of high-fat feeding. Thus, the beneficial effects of exercise on cardiovascular health do not appear to result merely from the reversal of the effects of poor dietary habits. Indeed, it is well-established that exercise training has beneficial effects, like improved endothelial function (Henderson et al. 2004; Padilla et al. 2006), that extend beyond its ability, or lack thereof, to modulate other known risk factors (Myers et al. 2002).

In summary, our results reveal that high-fat feeding increases FFA levels and inhibits the ACTH stress response in sedentary animals. Exercise training alone did not affect the ACTH stress response, but exercise training in combination with high-fat feeding exaggerates the ACTH response in the presence of elevated FFAs. Thus, exercise training reverses the effect of high-fat feeding on the ACTH stress response.

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Fig. 1.

Effect of high fat diet on HPA axis response to anesthesia and surgery. Basal ACTH (A) and cortisol (C) over a 24 h period in undisturbed animals ($n = 4$). ACTH (B) and cortisol (D) response to an acute stressor ($n = 8$). There was a significant interaction between diet and exercise for ACTH. High-fat diet decreased ACTH response in sedentary animals. Exercise training, in the high-fat fed animals, significantly increased the ACTH stress response. The cortisol stress response was unaffected by diet or exercise. Dagger (†) indicates $p < 0.05$ demonstrating effect of diet within activity group. Asterisk (*) indicates $p < 0.05$ demonstrating effect of exercise within dietary group.

Fig. 2.

The high-fat diet significantly increased serum cholesterol (A, $n = 5$) and FFAs (B, $n = 8$). Exercise training lowered FFAs in both dietary groups, but did not affect cholesterol. Section symbol (§) indicates $p < 0.05$ demonstrating main effect of high-fat diet. Asterisk (*) indicates $p < 0.05$ demonstrating effect of exercise within dietary group.

Fig. 3.

Effect of high-fat diet on glucose homeostasis. The high-fat diet resulted in significant increases in serum glucose (A), insulin (B), and IGF-1 (C). Exercise did not affect serum glucose, insulin, or IGF-1. Section symbol (§) indicates $p < 0.05$ demonstrating main effect of high-fat diet, $n = 8$.

Table 1
Group clinical chemistry profiles prior to initiation of diet and exercise.

	Normal diet		High-fat diet		p value
	Sedentary	Exercise	Sedentary	Exercise	
Alanine transaminase (U/L)	39.2±39	36.4±2.5	45.6±2.9	40±1.2	0.09
Albumin (g/dL)	4.3±0.1	4.4±0.2	4.3±0.1	4±0.2	0.52
Alkaline phosphatase (U/L)	68±3.8	68.6±11	72±9.9	83.2±12.1	0.67
Amylase (U/L)	1249±125	1503±243	1172±231	1364±190	0.68
Anion GAP	24±2.2	24.8±3.3	25.6±2.8	21±1.3	0.6
BUN (mg/dL)	14±1.1	15.6±1.4	14.4±1.5	15.6±1.5	0.79
CO ₂ (mEq/L)	22.6±0.9	20.6±2.1	21.6±2.2	24.2±0.9	0.53
Creatinine (mg/dL)	0.9±0	1.1±0	1.0±0	1.0±0.1	0.04*
Globulin	3.3±0.2	3.1±0.2	3.6±0.1	3.3±0.4	0.51
Total protein (g/dL)	7.6±0.2	7.5±0.4	7.8±0.2	7.3±0.4	0.62
Electrolytes					
Na (mEq/L)	140.8±2.3	138.2±5.4	143.4±2.5	138±1.7	0.63
K (mEq/L)	3.9±0.3	3.8±0.2	3.9±0.2	3.8±0.2	0.99
Cl (mEq/L)	98.2±1.5	96.4±3.7	100.2±2.2	96.8±1.1	0.67
Phosphate (mg/dL)	7.9±0.5	7.2±0.2	7.3±0.2	7.4±0.3	0.46
Ca (mg/dL)	10.4±0.2	10.6±0.2	10.6±0.1	10.5±0.2	0.81

Note: Values are presented as mean ± SE, n = 5/group. Asterisk (*) indicates p < 0.05 for one-way ANOVA.

Table 2
Effects of diet and exercise on body weight and exercise capacity.

	Normal diet		High-fat diet		p value	
	Sedentary	Exercise	Sedentary	Exercise	Exercise	Diet
Initial body weight (kg)	29±2.2	29.2±2.2	30.5±2.4	30.3±2.4	0.99	0.57
Final body weight (kg)	45.8±2.3	39.7±1	49.6±2.2	47.7±2	0.06	<0.01*
Heart weight (g)	191.7±6.4	206±5.4	195.4±6.1	234.6±8.7	<0.001*	0.02*
Heart—body weight ratio (g/kg)	4.2±0.1	5.2±0.2	4±0.1	4.9±0.1	<0.001*	0.07
Exercise capacity						
Pre (min)	26.2±1	28.4±0.9	25.9±1.1	25.3±1.5	0.51	0.14
Post (min)	26.6±0.8	39.7±1.4	22.4±1.5	33.3±0.7	<0.001*	<0.001*
Change (min)	0.4±0.8	11.3±1.7	-3.6±1.3	8.1±1.5	<0.001*	0.01*

Note: Values are presented as mean ± SE, n = 8/group. Pre, treadmill exercise test to exhaustion completed prior to initiation of diet or exercise training; post, treadmill exercise test to exhaustion completed after 16–20 weeks of diet and exercise treatments; change, difference in time to exhaustion from pre to post. Body and heart weights were collected after 16–20 weeks of diet and exercise treatments. Asterisk (*) indicates $p < 0.05$ for two-way ANOVA.

Table 3
Effects of diet and exercise on clinical chemistry profiles.

	Normal diet		High-fat diet		p value	
	Sedentary	Exercise	Sedentary	Exercise	Exercise	Interaction
Alanine transaminase (U/L)	39.2±1.7	49.8±2.9	32.8±2.3	34±2.7	0.028*	<0.001*
Albumin (g/dL)	3.7±0.1	3.7±0.2	3.4±0.1	3.3±0.1	0.78	0.012*
Alkaline phosphatase (U/L)	39.4±1.3	61.4±9.7	86.8±9.9	98.8±7.6	0.048*	<0.001*
Amylase (U/L)	944±110	1043±41	930±190	1118±129	0.28	0.82
Anion GAP	11±1.2	14±1.7	12.6±1.1	13.4±1	0.16	0.7
BUN (mg/dL)	21.2±3.6	23.6±2.7	12.2±2.1	11.6±1.4	0.73	<0.001*
CO ₂ (mEq/L)	30.8±1	29.6±0.4	29±0.8	29.6±0.2	0.67	0.22
Creatinine (mg/dL)	0.9±0.1	1.2±0.1	1.0±0	1.1±0.1	0.09	0.89
Globulin	2.8±0.3	3.2±0.2	3.4±0.3	3.4±0.1	0.3	0.09
Total protein (g/dL)	6.5±0.2	6.9±0.2	6.7±0.3	6.7±0.2	0.39	0.96
Electrolytes						
Na (mEq/L)	132.8±3	137.6±2.9	133±3.4	135.6±2.1	0.22	0.76
K (mEq/L)	3.6±0.2	3.9±0.2	3.9±0.2	3.9±0.1	0.39	0.58
Cl (mEq/L)	94.8±2.5	98±1.9	95.2±2	96.6±1.3	0.26	0.8
Phosphate (mg/dL)	5.4±0.2	5.9±0.2	5.9±0.2	6.4±0.3	0.047*	0.047*
Ca (mg/dL)	10.1±0.3	10.2±0.3	9.9±0.2	9.8±0.2	0.94	0.27

Note: Values are presented as mean ± SE, n = 5/group. Asterisk (*) indicates p < 0.05 for two-way ANOVA.