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ORIGINAL CONTRIBUTION

Exercise in ZDF rats does not attenuate weight gain, but prevents hyperglycemia concurrent with modulation of amino acid metabolism and AKT/mTOR activation in skeletal muscle

Olasunkanmi A. J. Adegoke · Holly E. Bates · Michael A. Kiraly · Mladen Vranic · Michael C. Riddell · Errol B. Marliss

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Abstract
Purpose Protein metabolism is altered in obesity, accompanied by elevated plasma amino acids (AA). Previously, we showed that exercise delayed progression to type 2 diabetes in obese ZDF rats with maintenance of β cell function and reduction in hyperglucocorticoidemia. We hypothesized that exercise would correct the abnormalities we found in circulating AA and other indices of skeletal muscle protein metabolism.
Methods Male obese prediabetic ZDF rats (7–10/group) were exercised (swimming) 1 h/day, 5 days/week from ages 6–19 weeks, and compared with age-matched obese sedentary and lean ZDF rats.
Results Food intake and weight gain were unaffected. Protein metabolism was altered in obese rats as evidenced by increased plasma concentrations of essential AA, and increased muscle phosphorylation (ph) of Akt^{ser473} (187 %), $mTOR^{ser2448}$ (140 %), eIF4E-binding protein 1 (4E-BP1) (111 %), and decreased formation of 4E-BP1*eIF4E complex (75 %, $0.01 \leq p \leq 0.05$ for all measures) in obese relative to lean rats. Exercise attenuated the increase in plasma essential AA concentrations and muscle Akt and mTOR phosphorylation. Exercise did not modify phosphorylation of S6K1, S6, and 4E-BP1, nor the formation of 4E-BP1*eIF4E complex, mRNA levels of ubiquitin or the ubiquitin ligase MAFbx. Positive correlations were observed between ph–Akt and fed circulating branched-chain AA ($r = 0.56$, $p = 0.008$), postprandial glucose ($r = 0.42$, $p = 0.04$) and glucose AUC during an IPGTT ($r = 0.44$, $p = 0.03$).
Conclusion Swimming exercise-induced attenuation of hyperglycemia in ZDF rats is independent of changes in body weight and could result in part from modulation of muscle AKT activation acting via alterations of systemic AA metabolism.

Keywords Protein metabolism · mRNA translation · Branched-chain amino acids · 4E-BP1 · Ubiquitin protein ligase

O. A. J. Adegoke · E. B. Marliss
Crabtree Nutrition Laboratories, Division of Endocrinology and Metabolism, Department of Medicine, McGill University Health Centre, Montreal, Canada

O. A. J. Adegoke (✉) · M. C. Riddell
School of Kinesiology and Health Science, York University, Toronto, ON M3J 1P3, Canada
e-mail: oadegoke@yorku.ca

H. E. Bates · M. A. Kiraly · M. Vranic
Departments of Physiology and Medicine, University of Toronto, Toronto, Canada

Present Address:
H. E. Bates
Trent University, Peterborough, ON, Canada

Present Address:
M. A. Kiraly
Department of Biology, Capilano University, North Vancouver, BC, Canada

Introduction

The disequilibrium between caloric intake and energy expenditure is the main cause of obesity, the most important predisposing factor for type 2 diabetes (T2DM). Insulin resistance accompanies obesity and T2DM. This condition is of particular significance in skeletal muscle as this insulin-responsive tissue contributes more than 40 % to whole-body energy metabolism. Impaired postabsorptive protein

synthesis and increased proteolysis are observed in obesity and diabetes [1–3]. We and others have shown that insulin resistance of whole-body protein metabolism accompanies that of glucose (and fat) metabolism in human obesity [1, 4, 5] and T2DM [6, 7]. These alterations are associated with elevated circulating substrate concentrations, including certain amino acids [1, 8, 9], which may be implicated in the metabolic sequelae.

Because obesity largely results from energy imbalance, exercise can be an important factor in limiting obesity development by increasing energy expenditure [10, 11]. Endurance exercise increases insulin sensitivity in patients with type 2 diabetes [12], suggesting that exercise might also regulate skeletal muscle protein metabolism. Exercise interventions prevent or delay many of the manifestations of diabetes [13, 14], but protective/preventive effects with respect to protein metabolism have not been clearly established. The demonstration of exercise effects requires that they be independent of changes in food intake and weight loss, which is challenging in human study design.

Male Zucker diabetic fatty (ZDF) fa/fa rats are characterized by obesity, progressive glucose intolerance, hyperglycemia, and hyperlipidemia [15], and impaired beta cell mass dynamics [16]. They have therefore been widely used as a model with similarities to human T2DM. Using this model, we showed that intermittent swimming delayed the onset of T2DM by mechanisms including increased insulin production and augmented insulin sensitivity [17, 18]. We therefore studied muscle insulin signaling and amino acid metabolism in these rats to determine whether this treatment affected skeletal muscle protein metabolism. Obese Zucker rats, the parental strain of the ZDF rats, have impaired protein metabolism [19, 20], but we are unaware of such studies in ZDF rats.

At the molecular level, the phosphoinositide-3-kinase (PI3K)/Akt/mammalian (mechanistic) target of rapamycin complex 1 (mTORC1) pathway is critical in regulating skeletal muscle protein metabolism and mass [21]. Specifically, mTORC1 mediates the effect of nutrients and insulin on protein synthesis. Activated mTORC1 stimulates mRNA translation initiation and protein synthesis by phosphorylating eukaryotic translation initiation factor 4E (eIF4E)-binding protein 1 (4E-BP1). This favors the dissociation of eIF4E from the inhibitory eIF4E*4E-BP1 complex and the formation of mRNA translation initiation-promoting eIF4E*eIF4G complex [22]. mTORC1 also promotes translation via the phosphorylation of the serine threonine kinase ribosomal protein S6 kinase 1 (S6K1 or p70S6K1) [23]. In addition, activation of the PI3K/Akt/mTORC1 pathway downregulates proteolysis by at least two mechanisms: mTORC1-dependent suppression of autophagy [24] and Akt-mediated downregulation of MAFbx and MuRF1 [21], two ubiquitin protein ligases that are critical to the ubiquitin-dependent proteolytic system in catabolic skeletal muscle.

The delay in the onset of diabetes in response to exercise led us to hypothesize that regular swimming exercise would improve skeletal muscle insulin signaling and promote translation initiation events within the muscle itself. Since muscle protein metabolism is the net result of regulation of both protein synthesis and breakdown, we also examined treatment effect on components of the ubiquitin-dependent proteolytic system. Finally, because increases in some individual plasma amino acids occur in obesity and T2DM [8, 9, 25–27], we reasoned that exercise would attenuate the levels of these nutrients. Such an attenuation might correct impairments in insulin signaling of glucose uptake and utilization, and of muscle protein synthesis and catabolism.

Materials and methods

Male 5-week-old obese ZDF (ZDF/Crl-*Lepr*^{fa}) and lean ZDF rats obtained from Charles River Laboratories (Wilmington, MA) were individually housed in opaque cages in temperature and humidity controlled rooms on a 0700–1900 hours light cycle and were fed Purina 5001 rat chow (LabDiet, St Louis MO). All experiments followed guidelines from the Canadian Council for Animal Care and were approved by the University of Toronto Animal Care Committee.

Rats were subjected to intermittent swimming exercise as detailed previously [18]. Briefly, obese ZDF rats were randomly divided into two groups (n = 7–10/group) and allowed to acclimatize for 1 week. From age 6 weeks, one group (Exercise) swam individually for 1 h in 60 cm diameter × 100 cm high cylindrical tanks, in ~35 °C water at a depth of 30–45 cm once daily between 0930 and 1130 hours, 5 days/week. During swimming, rats wore elastic chest bands onto which weights could be attached. No additional weight was added the first week, then 3 % of body weight was added the second week, followed by increases of 1 % each week to a maximum of 7 % body weight by week 12. All rats were transported together to the treatment room 30–60 min prior to the daily treatment. The 2nd group, nonexercised obese control rats (Obese CT), had food and water removed for the same periods. The 3rd group comprised lean ZDF (-/?) control rats (Lean) that were monitored daily for 13 weeks. In all groups, fasting blood samples were collected following 16–18 h food deprivation. Four days before euthanasia, and after an 18-h fast, an intraperitoneal glucose tolerance test (IPGTT) was performed (2 g/kg body weight) and blood sampled via a tail nick over a period of 2 h [18]. IPGTT was conducted ~24 h after an exercise regimen. Animals were

euthanized without overnight food withdrawal by decapitation 24 h after the last treatment, between 0830 and 1300 hours. Blood was collected and gastrocnemius muscles were removed, rapidly frozen in liquid N_2, and stored at −80 °C.

Analyses

Plasma individual amino acids (by HPLC), plasma glucose, and insulin (using rat standards) were measured as detailed previously [28, 29]. Muscle homogenization and immunoblotting were as described [28]. For any specific protein, electrophoresis and immunoblotting for all samples were done at the same time. To ensure treatments were arranged in a particular order (Figs. 1, 2, 3), bands were assembled from different section of blots analyzed at the same time and with the same image setting. For phosphorylated proteins (AKT, mTOR, S6K1, and ribosomal protein S6 (S6), results are expressed as a ratio of phosphorylated to the corresponding total protein.

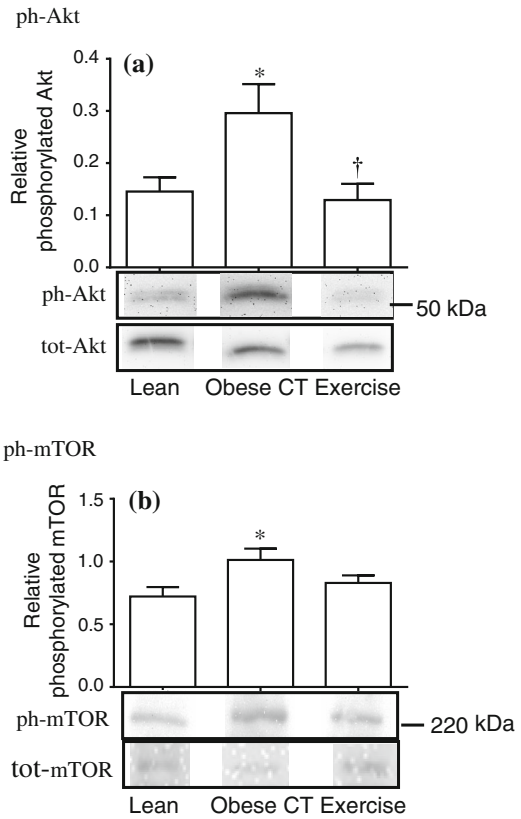

Fig. 1 Fed-state **a** Akt (ser473) and **b** mTOR (ser2448) phosphorylation in skeletal muscle of lean (Lean), obese (Obese CT), or obese rats subjected to intermittent swimming exercise (Exercise). Mean ± SEM (arbitrary units), $n = 7$–9 rats/group. *Significantly different from Lean; †significantly different from Obese CT ($p < 0.05$)

Muscle RNA was isolated, reverse-transcribed and the resulting cDNA used in quantitative PCR using the LightCycler FastStart DNA MasterPLUS SYBR Green1 and the LightCycler machine (Roche, Laval, QC, Canada) as described [28]. The following forward (F) and reverse (R) primers (5′-3′) were used: ubiquitin F: GGCAAGCA GCTGGAAGATGG; R: GAGCCCAGTGACACCATCG; MAFbx F: CAGCTGGATTGGAAGAAGATG, R: CTG GAAGGGCACTGACCAT. Each primer was designed to

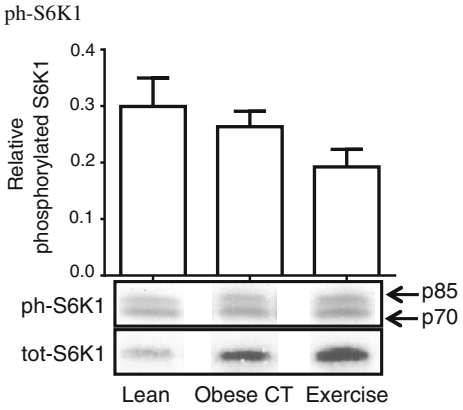

Fig. 2 Fed-state S6K1 (thr389) phosphorylation in skeletal muscle of lean (Lean), obese (Obese CT), or obese rats subjected to intermittent swimming exercise (Exercise). Mean ± SEM (Arbitrary Units), $n = 7$–9 rats/group

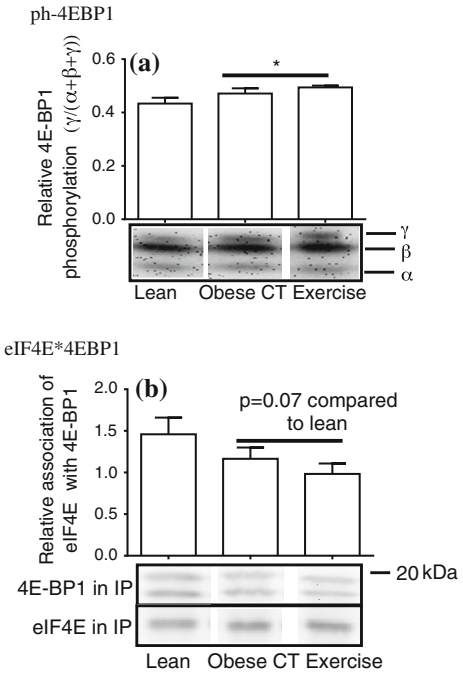

Fig. 3 Fed-state **a** 4E-BP1 phosphorylation and **b** association of eIF4E with 4E-BP1 in muscle of ZDF rats. Treatments are as in Fig. 1. Mean ± SEM (Arbitrary Units), $n = 7$–9 rats/group; *$p < 0.05$ significant effect of obesity

span intron–exon junctions to ensure genomic DNA would not be amplified. To correct for nonspecific effects of treatments on total mRNA, quantitative PCR was also carried out using porphobilinogen deaminase (PBGD) primers (F: GAAGTGGACCTGGTTGTTC; R: CCATCT GCAAACGGGAAAAC). Signals (crossover points) were converted to cDNA copies using standard curves conducted under identical run conditions. cDNA copies were expressed as a fraction of copies of PBGD cDNA.

Statistical analyses

Results are presented as mean ± SEM. Comparisons among groups were performed using one-way analysis of variance and post hoc analyses using Tukey's test. Significance was set at $p < 0.05$. Analyses were performed using SPSS 15.0 for Windows (SPSS Inc., Chicago, IL).

Results

Amelioration of hyperglycemia

As previously published [17, 18], the development of hyperglycemia was ameliorated with intermittent swimming exercise. The two obese ZDF groups (Obese CT and Exercise) had higher food intake and body weights than the lean group (Table 1). Importantly, neither food intake nor weight gain was different between the two obese groups. Compared to Lean, fasting and fed plasma glucose levels were significantly higher in Obese CT (Table 1). Exercise exerted a dramatic glucose lowering effect. Compared to Lean, Obese CT had impaired glucose tolerance (AUC for IPGTT); this was improved by exercise in spite of the fact that insulin AUC is comparable in Obese CT and Exercise.

Altered plasma amino acid profiles

Morning (fed) plasma concentrations of total nonessential amino acids (NEAA) and of aspartic acid, glutamine, and glycine were significantly lower in Obese CT compared to Lean and exercise exerted no effect (Table 2). Although concentrations of asparagine and citrulline in Obese CT were not different from Lean, exercise significantly reduced the levels of these amino acids by 38 and 23 %, respectively. Among the essential amino acids, there were no treatment effects on threonine, tryptophan and methionine. Except for histidine and lysine, whose concentrations were higher in Lean, the concentrations of the rest of the essential amino acids were higher in Obese CT. In particular, concentrations of individual and total branched-chain amino acids (BCAA) were 160–175 % of those observed in Lean. Notably, the concentrations of BCAA and of total essential amino acids in Exercise were markedly lower than in Obese CT. For example, concentrations of the BCAA were 25–32 % lower in Exercise (all $0.05 < p \leq 0.001$).

Changes in indicators of skeletal muscle protein metabolism

Exercise modified pathways that regulate skeletal muscle protein metabolism. Compared to Lean, muscle Akt (ser473) phosphorylation was higher in Obese CT (Fig. 1a). Exercise significantly suppressed Akt. Relative to Lean, mTOR phosphorylation was also higher in Obese CT (Fig. 1b). However, as with Akt, mTOR

Table 1 Body weight, plasma glucose, and insulin concentrations in the different experimental groups

	Lean	Obese CT	Exercise	Statistics (p values)	
				Genotype*	Exercise†
Body weight, g	365 ± 7	475 ± 19	463 ± 11	0.0001	ns
Food intake, g/d	20 ± 0.4	37 ± 2	32 ± 2	0.0001	ns
Fasting glucose, mM	4.5 ± 0.1	14.5 ± 2	7.1 ± 1	0.0001	0.01
Fed glucose, mM	5.5 ± 0.2	20.7 ± 1	9.4 ± 1	0.0001	0.0001
Fed insulin, ng/mL	2.5 ± 0.3	25.4 ± 6.0	35.6 ± 2.0	0.0001	ns
Glucose AUC[a]	1,289 ± 59	2,925 ± 207	2,052 ± 101	0.0001	0.01
Insulin AUC[a]	ND	1,297 ± 265	1,478 ± 219		ns

Mean ± SEM, $n = 7$–10

Data in this table are from the last points from time-course figures of a previous publication [17]

CT control, *ND* Not determined, *ns* no significant effect

* Obese CT compared to Lean; † compared to Obese CT

[a] AUC: area under the curve for insulin (Insulin AUC) or glucose (Glucose AUC) during an intraperitoneal glucose tolerance test (IPGTT)

Table 2 Fed plasma amino acid concentrations (μmol/L) in lean and obese ZDF rats

	Lean	Obese CT	Exercise	Statistics ($p <$)	
				Genotype*	Exercise†
Nonessential amino acids					
ALA	328 ± 6	313 ± 8	319 ± 4	ns	ns
ARG	220 ± 15	182 ± 10	160 ± 10	ns	ns
ASN	41 ± 3	45 ± 4	28 ± 2	ns	0.01
ASP	26 ± 3	16 ± 1	13 ± 1	0.001	ns
CIT	64 ± 3	76 ± 5	58 ± 2	ns	0.01
GLN	494 ± 25	290 ± 13	283 ± 15	0.0001	ns
GLU	163 ± 1	192 ± 7	169 ± 7	ns	ns
GLY	289 ± 22	121 ± 11	107 ± 10	0.0001	ns
ORN	80 ± 5	105 ± 9	86 ± 11	ns	ns
SER	177 ± 18	121 ± 11	100 ± 4	0.05	ns
TAU	231 ± 22	272 ± 29	265 ± 22	ns	ns
TYR	101 ± 8	86 ± 3	85 ± 2	ns	ns
Essential amino acids					
ILE	87 ± 3	153 ± 11	106 ± 4	0.0001	0.001
LEU	148 ± 5	252 ± 21	171 ± 6	0.0001	0.001
VAL	193 ± 7	308 ± 18	231 ± 7	0.0001	0.001
HIS	66 ± 3	50 ± 1	46 ± 1	0.001	ns
THR	221 ± 17	184 ± 12	157 ± 7	ns	ns
TRP	119 ± 6	108 ± 5	117 ± 3	ns	ns
PHE	69 ± 2	78 ± 3	69 ± 2	0.05	0.05
MET	48 ± 3	44 ± 3	40 ± 1	ns	ns
LYS	445 ± 28	369 ± 16	321 ± 15	0.05	ns
BCAA	428 ± 16	713 ± 49	509 ± 17	0.0001	0.001
EAA	1,396 ± 68	1,547 ± 78	1,259 ± 37	ns	0.05
NEAA	2,212 ± 115	1,819 ± 53	1,672 ± 41	0.01	ns
Total	3,609 ± 178	3,366 ± 127	2,930 ± 69	ns	ns

Mean ± SEM, $n = 7$–9 per group

* Obese CT compared to Lean; † compared to Obese CT

phosphorylation in Exercise tended to be lower ($p = 0.1$). There were no effects of obesity or exercise on phosphorylation of S6K1 (Fig. 2) or on that of its substrate, ribosomal protein S6 (ratio of phosphorylated S6 to total S6: 0.88 ± 0.2, 2.83 ± 1.3 and 0.78 ± 0.2 for Lean, Obese CT, and Exercise, respectively; $p > 0.05$). Because exercise had no apparent effect on phosphorylation of 4E-BP1 or its binding to eIF4E, data for the two obese groups were combined to allow for a better comparison between obese and lean groups. Phosphorylation of 4E-BP1 was higher in the obese groups compared to Lean (Fig. 3a), along with a corresponding reduction in the inhibitory eIF4E*4E-BP1 complex (Fig. 3b). There were no treatment effects on mRNA expression of ubiquitin or of Atrogin1/MAFbx in skeletal muscle (Table 3). Significant positive correlations were observed between fed-state glucose and plasma total EAA ($r = 0.44$, $p = 0.04$, figure not shown) and BCAA (Fig. 4a). Likewise, relative phosphorylation of Akt correlated positively and significantly with plasma total EAA ($r = 0.53$, $p = 0.01$, figure not shown), BCAA (Fig. 4b), glucose (Fig. 4c) and glucose AUC (Fig. 4d).

Table 3 Ubiquitin and MAFbx mRNA expression (arbitrary units) in muscle of ZDF rats

	Lean	Obese CT	Exercise
Ubiquitin	155 ± 12	136 ± 14	128 ± 1
MAFbx	19 ± 6	24 ± 5	23 ± 7

Mean ± SEM, $n = 7$–9

Discussion

We previously demonstrated that intermittent exercise delayed the development of diabetes in obese ZDF rats. These effects are reflected in attenuated fed and fasting hyperglycemia, increased insulin secretion, and improvements in intraperitoneal glucose tolerance (Table 1; [17, 18]). Here, we show that although exercise did not affect

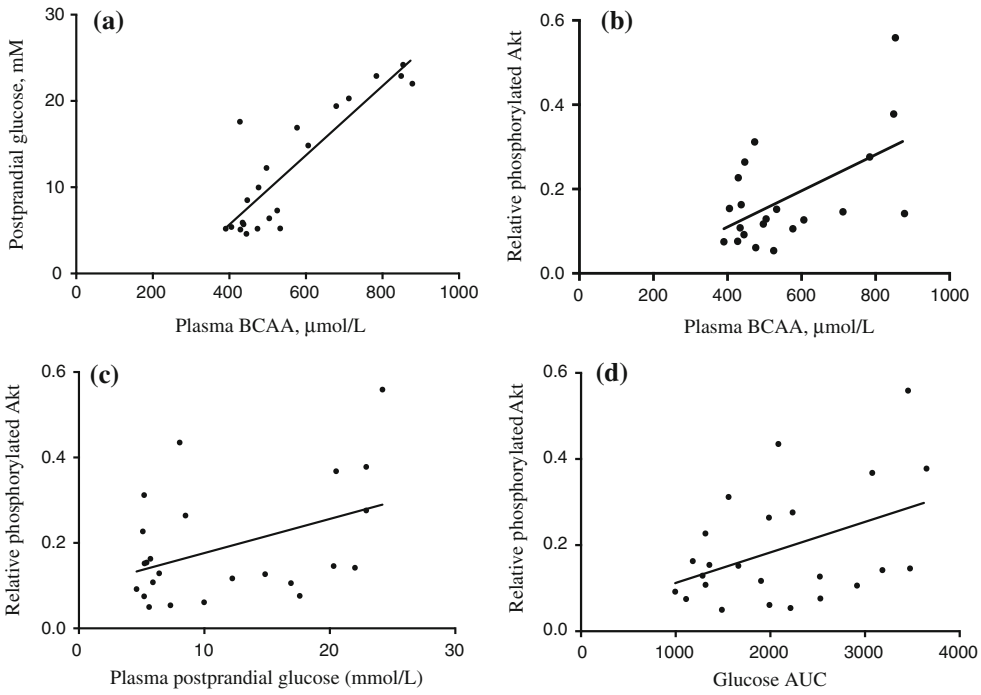

Fig. 4 Correlation of **a** plasma BCAA and postprandial glucose ($r = 0.88$, $p < 0.0001$), and of fed-state muscle phosphorylated Akt (arbitrary units) with **b** plasma BCAA (µmol/L; $r = 0.56$; $p = 0.008$), **c** postprandial plasma glucose (mM, $r = 0.42$; $p = 0.038$), and **d** Glucose AUC ($r = 0.44$, $p = 0.034$)

food intake or body weight, it attenuated the effects of diabetes on plasma amino acids, particularly BCAA, in parallel with reduced phosphorylation of skeletal muscle Akt and mTOR. The changes in circulating amino acid levels are concurrent with the attenuation in diabetes development, like many of the other changes in metabolism that we have already documented. Our data suggest that modulation of circulating amino acids and muscle AKT may represent an additional mechanism for the effect of exercise.

Plasma BCAA in particular, like most amino acids in general, are abnormally high in fed male ZDF rats as diabetes develops [30]. Fasted plasma amino acids are also higher in obese individuals [1, 26, 27]. These changes in BCAA profile are noteworthy since elevated amino acids can attenuate skeletal muscle insulin-mediated glucose uptake in healthy, lean individuals [31, 32]. However, our recent data in adults with T2DM indicate that hyperaminoacidemia at postprandial levels does not dampen insulin signaling nor impact the preexisting insulin resistance of glucose uptake [29]. Nevertheless, in the current study, improvements in fasted and fed glycemia and glucose tolerance observed in the exercise group occurred in parallel with significant reductions in plasma amino acid levels. In a study comparing obese T2DM and obese nondiabetic African American women, leucine and valine concentrations are higher in the T2DM group and are positively correlated with HbA1c [33]. In another metabolomics study in T2DM, improvement in glucose homeostasis with different weight-reduction interventions is related to circulating BCAA and their metabolites [34]. Whether elevations in circulating levels of BCAA and their metabolites worsen insulin resistance or merely reflect the severity of the condition is currently unclear [25]. Insofar as concentrations of circulating individual amino acids affect rates of presentation to the liver, their reduction with exercise might have led to fewer gluconeogenic amino acids (except alanine) being taken up for gluconeogenesis, a process that is increased in diabetes. Accordingly, the relationships between plasma amino acids and glucose homeostasis, as well as the relative significance of different body tissues as the source of those metabolites [35], merit further cause–effect studies.

In the exercise group, the reduction in plasma AA was not due to reduced food intake or a change in body weight (Table 1). This suggests increased fat utilization and/or higher energy efficiency in this group. The reduction in plasma amino acid concentrations in response to physical activity could be due to increased skeletal muscle protein synthesis and/or reduced proteolysis. Increased energy expenditure in the absence of a corresponding increase in caloric intake might also suggest increased oxidation of proteolysis-derived amino acids. Using the ZDF model, we have demonstrated that voluntary wheel-running exercise prevents the hypothalamic–pituitary–adrenal hyperactivity largely via decreased adrenal sensitivity to ACTH, thus decreasing plasma corticosterone [36]. Moreover, decreased hepatic and circulating inflammatory markers (including the cytokine IL-6) occur with exercise [37]. These previous observations may explain the reduced

circulating amino acids in our swimming exercise model, since glucocorticoids and IL-6 are activators of muscle proteolysis [38]. However, we did not observe any changes in the mRNA expression of components of the ubiquitin proteolytic system examined. We cannot rule out the possibility that proteolysis might be modified by events beyond gene expression and/or via pathways other than the ubiquitin system.

The mechanisms of the suppressive effect of amino acids on insulin-mediated glucose uptake have been the subject of recent studies. In response to insulin and/amino acids, activation of mTORC1 leads to the activation of S6K1 [39]. This kinase, in addition to its role in mRNA translation initiation, also phosphorylates insulin receptor substrate 1 on serine residues, which leads to inhibition of downstream signaling [39]. Although we did not measure muscle intracellular amino acid concentrations, plasma concentrations of amino acids, especially the BCAA, reflect the intracellular levels [40]. Others have shown that whole leg muscle leucine concentration in obese rats is >2X that found in lean animals [41]. During exercise, there is increased amino acid release from skeletal muscle [42, 43]. Furthermore, branched-chain amino acid α-keto acid dehydrogenase complex is activated by exercise [44, 45]. These observations would be consistent with lower intracellular leucine (and other BCAA). As a consequence, reduced phosphorylation of S6K1 in response to exercise would be expected. However, phosphorylation of S6K1 or of S6 (a substrate of S6K1) in obese animals was not affected by treatment. Collectively, our data suggest that in this model, elevated plasma amino acid levels do not modulate S6K1 activation and that over-activation of S6K1 might not contribute to the development of insulin resistance in these animals. Alternatively, over-activation of S6K1 might be an early event that occurred in the development of insulin resistance, which would not be detected in our study as muscles were sampled only at the end of the 13-week treatment period. Thus, in follow-up studies, it would be interesting to examine whether treatment with rapamycin or other mTORC1/S6K1 inhibitors would attenuate insulin resistance in these animals.

Our finding of elevated Akt phosphorylation in the muscle of insulin resistant rats was surprising, given that phosphorylation at this residue (ser473) is normally associated with activation of the kinase and raises the question of whether this link is causative or associative. Other studies have reported discordances between insulin sensitivity and Akt phosphorylation in muscle [46, 47]. Systemic inflammation can activate muscle Akt [48] and muscle from mice made obese by high fat feeding show increased basal Akt serine 473 phosphorylation [49]. Collectively, these data implicate over-activation of Akt in the pathogenesis of diet- and genetic-defect induced obesity and T2DM. Our study demonstrates the novel finding that moderate endurance exercise lowers Akt hyperphosphorylation in the face of higher circulating insulin levels. The suppression of AKT is likely related to the reduced inflammation we demonstrated in exercised obese ZDF rats [37]. Interestingly, the modulation of Akt and mTOR phosphorylation occurred in parallel with reduced plasma amino acid levels. In addition to activating mTORC1 [39], amino acids can also activate mTORC2 (an activator of Akt) [50] and Akt itself [51], which can lead to further activation of mTORC1 and impairment of insulin signaling. Indeed, we observed strong positive correlations between phosphorylated Akt and plasma essential (especially branched-chain) amino acids, postprandial glucose and glucose area under the curve (Fig. 4). An important future study would be to examine the influence of drug/genetic inhibition of muscle AKT on the response of these animals to exercise.

A limitation of our study is that we did not measure IRS1 phosphorylation (especially on inhibitory serine residues), which would be useful in assessing whether some effects of exercise are mediated via modification of those residues. Also, it would have been useful to have measures of protein synthesis and breakdown. However, changes in signaling proteins (for example, 4E-BP1 phosphorylation and interaction with eIF4E), and MAFbx expression, often occur in parallel with changes in protein synthesis and breakdown, respectively. Although the ZDF rat has been useful in gaining many insights into T2DM pathophysiology and interventions, extrapolations to other diabetic syndromes must take account of its genetic defect in leptin signaling.

In conclusion, we showed that hyperglycemia and hyperinsulinemia occur concurrent with elevated plasma essential amino acids and increased phosphorylation of Akt in skeletal muscle of ZDF rats. Exercise-induced attenuation of hyperglycemia in ZDF rats could result in part from modulation of muscle AKT activation resulting from alterations of systemic AA metabolism and, by implication, mTORC2 activity. The exercise protocol employed appears to be effective in controlling these metabolic abnormalities and in delaying the progression to T2DM.

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Conflict of interest The authors declare there is no conflict of interest in relation to this work.

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