

# **The effects of amino-acid supplementation, diet and chronic exercise training on glucose tolerance in Sprague-Dawley rats**

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## **Abstract**

The purpose of this study was to look at the effects of an amino-acid mixture, high-fat feeding and exercise on blood glucose and plasma insulin levels in skeletal muscle of female Sprague-Dawley rats. Rats were either fed a standard-diet only (SD-CON), a standard-diet plus a pre-established amino-acid mixture (CON-AA), a high-fat diet only (HF-CON), a high-fat diet plus a pre-established amino-acid mixture (HF-AA) or a high-fat diet, plus the amino-acid mixture coupled with chronic exercise training (HF-AA-EX). An Oral Glucose Tolerance Test (OGTT) was conducted after two- and four-weeks of treatment to assess blood glucose and plasma insulin levels. Rats were gavaged with a glucose bolus after a 12-h fast, and then blood was collected from the tail vein prior to, and 15, 30, 60, and 120 minutes after gavage. At the end of four weeks, rats were presented with an amino acid challenge and hind-limb muscles were removed for analysis of total GLUT-4 content. Results indicate that amino-acids are effective in lowering blood glucose in rats given either a standard diet or a high-fat diet despite elevated plasma insulin levels in rats fed a high-fat diet. Additionally, an exercise treatment coupled with amino-acids is more effective in lowering blood glucose than amino-acids alone in rats fed a high-fat diet. The addition of an exercise treatment may also help in the utilization of insulin by skeletal muscle cells. These results suggest that amino-acids and exercise may help control blood glucose levels thereby possibly alleviating some of the symptoms associated with insulin resistance.

## **1. Introduction**

Glucose homeostasis is the body's maintenance of normal glucose levels in the blood. Skeletal muscle is the major tissue associated with glucose metabolism, making up 75% of whole body insulin-stimulated glucose uptake (Corcoran et al., 2006). When glucose levels rise in the blood stream, insulin is secreted from the beta cells of the pancreas. In a healthy individual, insulin binds to insulin receptors on the plasma membrane of skeletal muscle, causing a cascade of effects to translocate a glucose transporter, GLUT-4, from its intracellular storage compartment to the plasma membrane (Watson & Pessin, 2001). Glucose can then be brought into the cell and blood glucose levels returned to a homeostatic level. Insulin resistance is a condition in which the body no longer responds normally to the hormone insulin. When an individual is insulin resistant, the signal transduction pathway, distal to the insulin receptor (Kleinert et al., 2011) no longer functions as it should, and fewer GLUT-4 transporters translocate to the cell membrane.

It is commonly known that a high-fat diet coupled with a sedentary lifestyle puts one at a greater risk for developing metabolic syndrome, which includes insulin resistance, and is associated with type 2 diabetes as well as other cardiovascular risks. Studies have repeatedly shown that a high-fat diet induces skeletal muscle insulin resistance in rats (Han et al., 1997; Kim et al., 2000; Liao & Xu, 2011; Storlein et al., 1986; Tremblay et al., 2001; Zierath et al., 1997). Rats fed a high-fat diet exhibit increased abdominal fat, increased plasma insulin, hyperglycemia, increased total cholesterol, LDL, and triglycerides (Han et al., 1997). Studies suggest that this defect arises from impaired activation of insulin receptor substrate 1 (IRS-1) associated phosphatidylinositol 3'-kinase (PI3K) activity (Tremblay et al., 2001). If PI3K is inactivated, everything downstream of it is shut off, and GLUT-4 can no longer translocate to the plasma membrane. Liao and Xu (2011) found that rats fed a short-term high-fat diet showed increased basal ribosomal protein S6 kinase1 (S6K1). This is part of the mammalian target of rapamycin (mTOR)/S6K1 pathway, which is activated by the signaling protein Akt. They theorized that chronic increased activation of this pathway led to skeletal muscle insulin resistance due to the negative feedback loop which inactivates insulin receptor substrate 1 (IRS-1), and this in turn, as others observed, impairs PI3K.

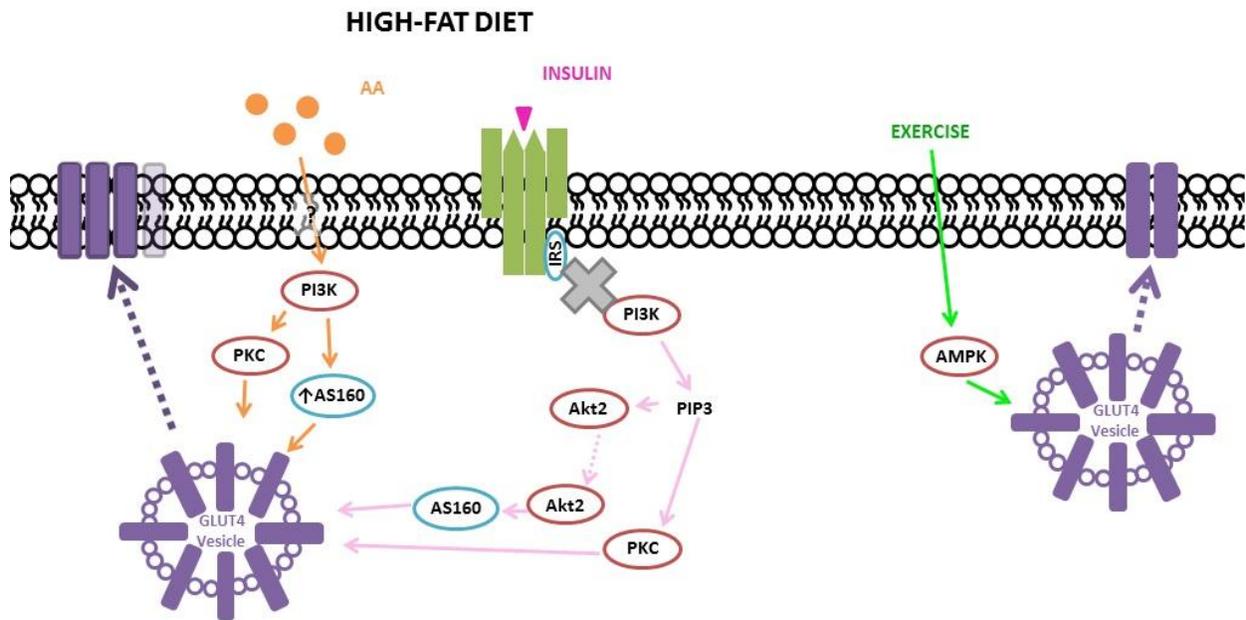
It is well-known that there are at least two distinct pools of GLUT-4 inside the cell, one which responds to insulin and one which responds to muscle contraction (Goodyear & Kahn, 1998). Exercise induces GLUT-4 translocation independently of PI3K through the adenosine 5'-monophosphate-activated protein kinase pathway (AMPK) (Liu et al., 2005). AMPK acts as an energy sensor which leads to contraction stimulated glucose transport in skeletal muscle through GLUT-4, independently of the insulin signaling pathway (Doi et al., 2005). Thus exercise has been widely studied in rats fed a high-fat diet, and so made insulin resistant, to evaluate the effects of glucose transport utilizing the AMPK pathway. Research has confirmed that exercise can prevent insulin resistance associated with the consumption of a high-fat diet (Friedrichsen et al., 2006; Liao & Xu, 2011; Sano et al., 2011). Studies looking at rats fed a high-fat diet and exercise treatments have found that exercise increases GLUT-4 expression and glucose transport rate (Jung & Kang, 2010) as well as increases total GLUT-4 content (Zhang et al., 2009).

Research has also focused on the use of branched chain amino acids (BCAA) as a blood glucose lowering therapy. Doi (2003, 2005) found that isoleucine stimulated glucose uptake in rat skeletal muscle in vivo through a mechanism independent of insulin and independent of AMPK. The decrease in blood glucose levels utilized PI3K and protein kinase C (PKC) phosphorylation, without an increase in plasma insulin (Doi et al., 2003; Doi et al., 2005). Nishitani (2005) found that leucine and isoleucine promoted GLUT-4 translocation without the activation of Akt, an important downstream reaction in insulin-stimulated GLUT-4 translocation. Further research found that a mixture of several BCAA was more effective at lowering blood glucose than one amino acid alone, and that this glucose transport was associated with increased AS-160 (rab GTP-ase activating protein) phosphorylation (Bernard et al., 2011; Kleinert et al., 2011).

Research on the various pathways which induce GLUT-4 translocation suggests that all three mechanisms, insulin, exercise, and amino-acids, utilize at least partially separate pathways (Fig. 1). Therefore, the purpose of this study was to examine the effects of an amino-acid mixture, high-fat feeding, and exercise on blood glucose and plasma insulin levels in rat skeletal muscle.

### 1.1 Rational

This investigation was designed as a pilot study to determine if 1) chronic amino acid supplementation improved glucose tolerance in both normal and high-fat fed rats and 2) if the combined effects of amino acids and exercise were additive. Due to limited resources available during the Summer Research Program, three groups were studied, a high-fat diet control, high-fat diet plus amino-acid supplementation, and high-fat diet, amino-acid supplementation and exercise training. It is well known throughout the literature that exercise improves glucose tolerance in healthy, insulin resistant, normal-fed and high-fat fed rats. Thus, for the purposes of this pilot study, we did not include a high-fat diet exercise or a control exercise group. These groups will be added at a later date. Furthermore, information presented in this report is from data collected during January Term and during the Summer Research Program. Both sets of data will be presented together in order to provide a more thorough story of how amino acid supplementation, diet and exercise impact glucose tolerance. Our January 2012 study provides data on rats fed a standard diet: a control group, as well as rats fed a standard diet and given the amino-acid supplement. These data provide a reference for non-insulin resistant blood glucose levels and plasma insulin. No known research, prior to the current study, has looked at the effects of amino-acids in rats fed a high-fat diet. Data from this study are to be used as pilot data for further research in a study which includes all control and treatment groups.



**Fig. 1.** Outline of the three main pathways for GLUT-4 translocation in skeletal muscle. Rats are made insulin-resistant through a high-fat diet, which inactivates the IRS-PI3K mechanism and turns off all downstream signals. Exercise utilizes its own GLUT-4 pool through the AMPK pathway. Amino-acids share characteristics with the insulin-signaling pathway and thus likely share the same pool of GLUT-4. Amino-acids have been shown to activate PI3K, PKC, and AS160. AA: amino-acids; Akt2: protein kinase Akt-2 (also known as PKB, protein kinase B) AMPK: adenosine 5'-monophosphate-activated protein kinase pathway; AS160: rab GTPase-activating protein; IRS: insulin receptor substrate-1; PI3K: phosphatidylinositol 3'-kinase; PIP3: phosphatidylinositol 3,4,5-triphosphate; PKC: protein kinase C.

## 2. Materials and Methods

### 2.1 *Animal Care and Housing*

Animal handling and care was according to standard animal handling protocol and animals were cared for to maintain the least stressful environment possible. Thirty female ~7 week old Sprague-Dawley rats were obtained from Harlan (Indianapolis, IN). Rats were randomly assigned to one of five groups: standard diet control (SD-CON, n=6), standard diet plus a pre-established amino-acid mixture with increased isoleucine concentration (SD-AA, n=6), high-fat diet control (HF-CON, n=6) (data provided by Dr. Liao), high-fat diet plus the amino-acid mixture (HF-AA, n=6) or high-fat diet plus the amino-acid mixture and exercise treatment (HF-AA-EX, n=6). Rats were housed two to a cage and given access to the respective chow and water ad libitum. The temperature of the animal room was maintained at 21°C with a 12-h light/dark cycle. Rats were kept for four weeks. A day of acclimation was given to the rats before the amino acid mixture was administered. Exercise treatment was conducted after two days of acclimation, and rats were given a week to become familiarized with the exercise treatment.

### 2.2 *Experimental Protocol*

The 60% high-fat diet, with fat coming predominantly from hydrogenated coconut oil, was obtained from Dyets, Inc. (#101920 AIN-93G, Bethlehem, PA). All other treatment groups not receiving a high-fat diet were given standard laboratory chow from LabDiet (Prolab RMH 1800 5LL2, Brentwood, MO). After one day of acclimation, rats receiving the amino-acid treatment were given an amino-acid mixture used in previous studies investigating glucose tolerance (Bernard et al., 2011; Bernard et al., 2012; Kleinert et al., 2011; Wang et al., 2012). The amino acid supplement was mixed into their drinking water and consisted of 8% amino-acids (cysteine, methionine, valine, isoleucine, and leucine) and 2% sucrose. For every 50mL of water, a specific amount of sucrose and amino acids was added and the drink was then brought to volume. The drink was divided among the cages, totaling approximately 50ml per day per cage. Rats in the exercise treatment group were given one week to become familiarized with swimming in barrels. Barrels measured 24 inches in diameter and 36 inches in height. Water in the barrels was maintained at ~35°C. Rats swam every two to three days, totaling ten exercise sessions over the course of the four weeks. After the first week, weight was attached to the tail to reduce rat buoyancy and increase the work-load. Rats swam with up to 8% body-weight attached to the tail, for up to one hour, according to ability. Rats unable to swim for the full hour were taken out and either had their weight reduced or swimming time reduced. This training protocol had been shown previously to provide a sufficient exercise stimulus to improve glucose tolerance (Hara et al., 2011; Morrison et al., 2008)

An Oral Glucose Tolerance Test (OGTT) was administered after two- and four-weeks of treatment. Rats were fasted for 12-h prior to the beginning of the OGTT. Blood samples were obtained by cutting the tip of the rat tail and taking 0.5ml of blood from the tail vein. Basal blood glucose levels were first obtained and then rats were gavaged with a 22.5% glucose solution (8ml/kg). Blood samples were collected at 15', 30', 60', and 120' after gavage. Blood was collected in a microcentrifuge tube containing EDTA. After the four-

week OGTT, rats were given two days to recover and then underwent surgery to remove the hind-limb muscles. Rats were gavaged (8ml/kg) with amino-acid mixture, prepared as described above, then anesthetized with an intraperitoneal injection of pentobarbital sodium (65mg/kg) 45 min after supplementation. The soleus, quadriceps, and gastrocnemius were excised and then rats were euthanized by cardiac injection of pentobarbital sodium (65 mg/kg). Excised muscle was freeze-dried in liquid nitrogen and then stored at -80°C for further analysis.

### 2.3 Blood Analysis

To measure blood glucose for the OGTT, a drop of blood was immediately analyzed using a glucose analyzer (One Touch Ultra 2; LifeScan, Milpitas, CA). Test tubes containing the blood sample and EDTA were centrifuged at 14,000 rpm for 10 minutes at room temperature. The tubes were stored at -80°C for further analysis. Plasma insulin was measured by ELISA according to the manufacturer's instructions (Alpco, Salem, NH).

### 2.6 Statistical Analysis

A two-way ANOVA was performed on the blood glucose and plasma insulin data (treatment × time). After finding an overall significance between treatment groups, a LSD post-hoc test was used to find statistical significance between means ( $p < 0.05$ ). Statistical analysis was conducted using SPSS software (SPSS, Chicago, IL). All values are expressed as mean ± SE.

## 3. Results

### 3.1 Animal Characteristics

HF-CON weight gain was significantly higher than all other treatment groups. No other significant differences in weight gain between the treatment groups were observed (Table 1).

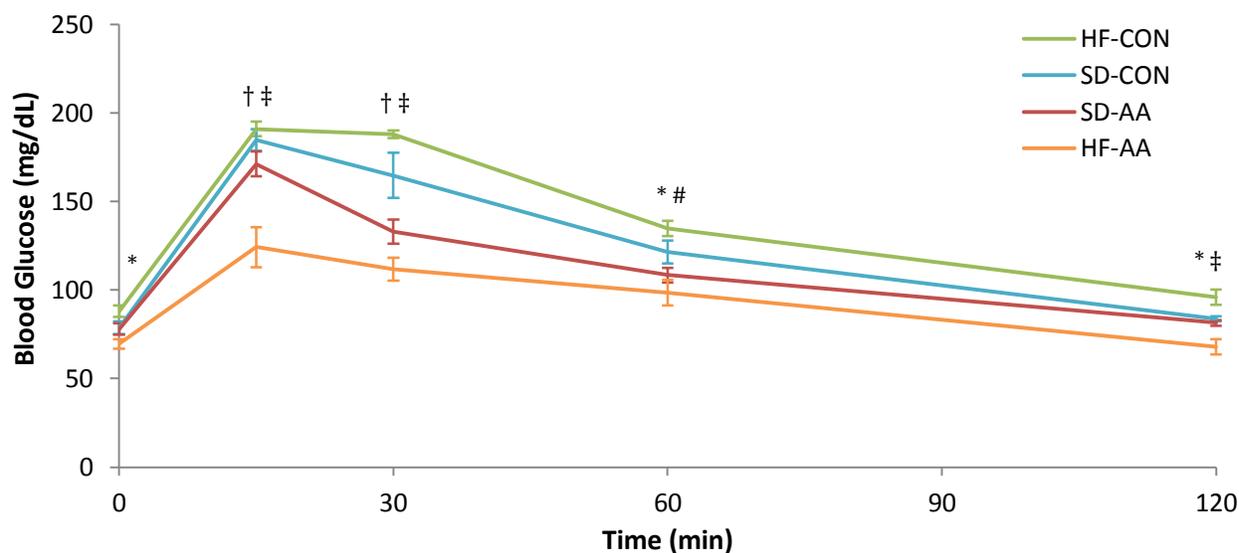
Group	Starting Weight (g)	Ending Weight (g)	Weight Gain (g)
SD-CON	125.5 ± 1.9	186.3 ± 5.4	60.8 ± 4.8
SD-AA	124.8 ± 1.9	189.2 ± 2.8	64.3 ± 3.3
HF-CON	122.2 ± 2.4	193.3 ± 9.9	71.1 ± 6.9
HF-AA	129 ± 3	187 ± 4	58 ± 2
HF-AA-EX	125 ± 2	182 ± 3	57 ± 2

**Table 1.** Body mass measurements at start and end of experiment, including overall weight gain over four weeks. Values are mean ± SE.

### 3.2 OGTT and Plasma Insulin

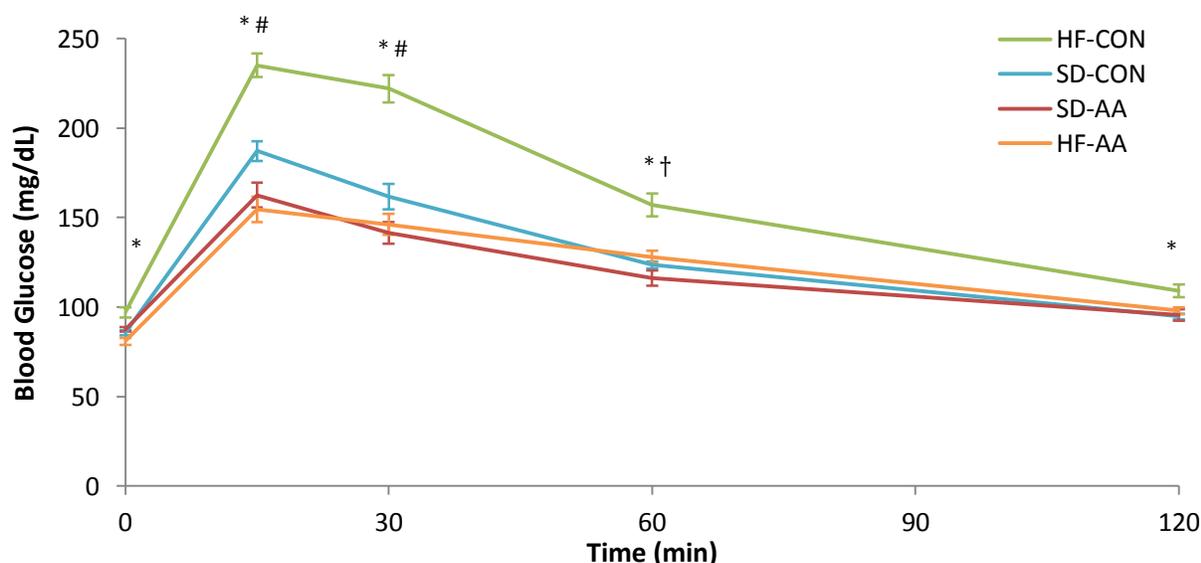
Blood glucose levels were determined during the oral glucose tolerance test (OGTT) following a 12-h fast at both two and four weeks. Blood was collected from the tail vein before and 15, 30, 60, and 120 minutes after gavage. Plasma insulin was determined from the OGTT.

The first comparison of blood glucose levels addressed is between the control groups and amino-acid treatment, including High-Fat Diet Control (HF-CON), Standard-Diet Control (SD-CON), Standard-Diet and Amino-Acid Mixture (SD-AA) and High-Fat Diet and Amino-Acid Mixture (HF-AA). During the first OGTT taken at 2 weeks (Fig. 2), there was a significant difference at 0 minutes between HF-CON and all other treatment groups. After fifteen and thirty minutes, blood glucose was significantly lower for both HF-AA and SD-AA compared to the control groups, and HF-AA was significantly lower than SD-AA. At one hour, SD-CON was significantly lower than HF-CON, but both control groups continued to have a significantly higher blood glucose level than the amino-acid treatments. The two amino-acid treatments were not significantly different from each other. As blood glucose levels returned to homeostatic levels after two hours, HF-CON remained significantly higher than all other groups, while HF-AA remained significantly lower than all other treatment groups.



**Fig. 2. Oral Glucose Tolerance Test (OGTT) at 2 weeks.** Sprague-Dawley rats in HF-CON (High-Fat Diet Control), SD-CON (Standard-Diet Control), SD-AA (Standard-Diet + Amino-acid Mixture), HF-AA (High-Fat Diet + Amino-acid Mixture) treatments were gavaged with glucose. Blood was collected from the tail vein before gavage and 15, 30, 60, and 120 minutes after gavage. Values are mean  $\pm$  SE. \* $p$ <0.05 HF-CON vs. all other treatments. # $p$ <0.05 SD-CON vs. all other treatments. † $p$ <0.05 SD-AA vs. all other treatments. ‡ $p$ <0.05 HF-AA vs. all other treatments.

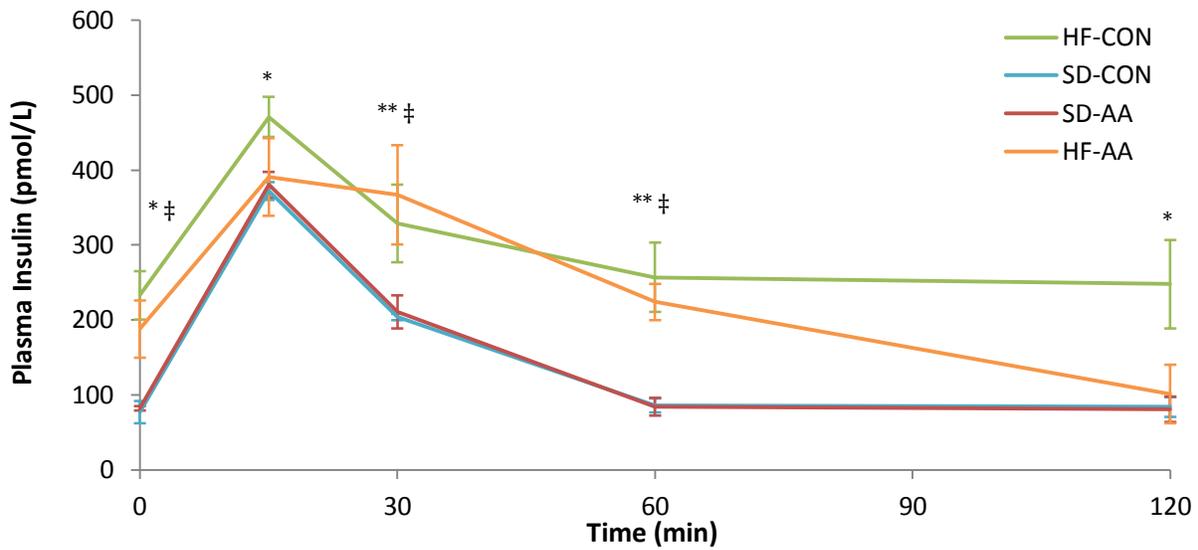
The final OGTT after four weeks (Fig. 3) showed a significantly higher blood glucose level for HF-CON compared to all other treatments at each time point. The SD-CON group was significantly higher than the two amino-acid treatments at 15 and 30 minutes. At 60 minutes, SD-AA blood glucose was significantly lower than HF-AA. After two hours, only HF-CON blood glucose levels remained elevated while the other treatment groups returned close to baseline levels without a significant difference between the three.



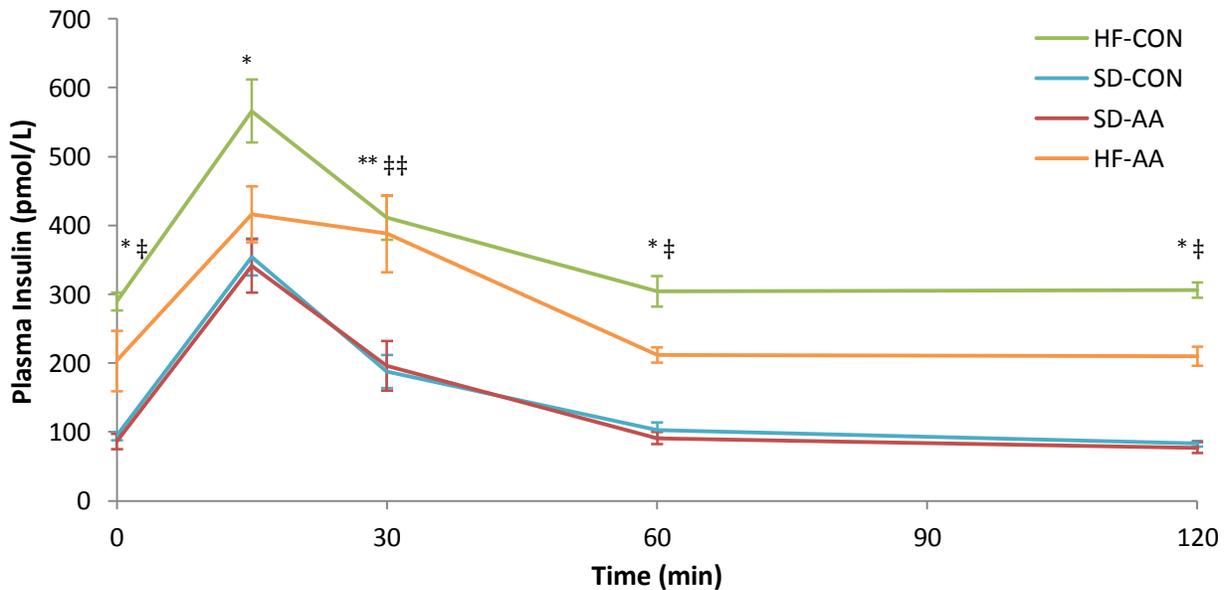
**Fig. 3. Oral Glucose Tolerance Test (OGTT) at 4 weeks.** Sprague-Dawley rats in HF-CON (High-Fat Diet Control), SD-CON (Standard-Diet Control), SD-AA (Standard-Diet + Amino-acid Mixture), HF-AA (High-Fat Diet + Amino-acid Mixture) treatments were gavaged with glucose. Blood was collected from the tail vein before gavage and 15, 30, 60, and 120 minutes after gavage. Values are mean  $\pm$  SE. \* $p$ <0.05 HF-CON vs. all other treatments. # $p$ <0.05 SD-CON vs. all other treatments. † $p$ <0.05 SD-AA vs. HF-AA.

In the 2-week OGTT, plasma insulin levels (Fig. 4) were significantly elevated for HF-CON compared to standard-diet treatments at all time points. The HF-AA group was significantly lower than HF-CON only after two hours. Both SD groups had significantly lower levels of plasma insulin than the high-fat groups at baseline and at 30 and 60 minutes. At 15 and 120 minutes, HF-AA insulin levels were not significantly higher than that of the standard-diet groups.

After four weeks, plasma insulin levels (Fig. 5) for HF-CON peaked much higher at 15 minutes and remained elevated compared with after two weeks. As seen in the 2 week OGTT, after four weeks HF-CON was significantly higher than the standard-diet groups across the two hours. The HF-AA treatment group had significantly lower plasma insulin levels than HF-CON at all time points except 30 minutes, but had significantly higher plasma insulin levels than the standard-diet treatment groups at 0, 30, 60 and 120 minutes. There was no significant difference between plasma insulin levels of the standard-diet treatment groups.

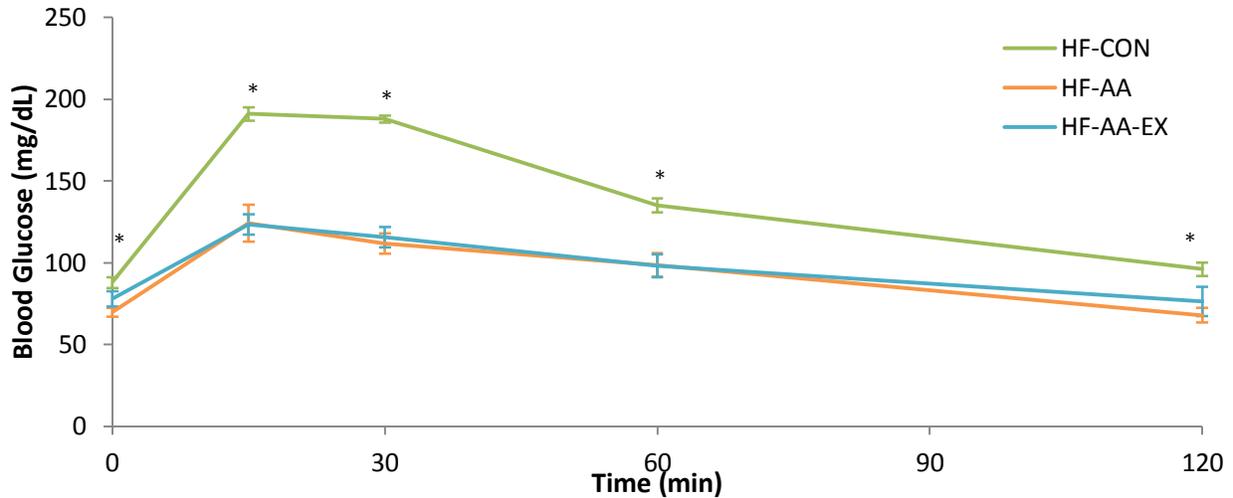


**Fig. 4. Plasma insulin during OGTT at 2 weeks.** Sprague-Dawley rats in HF-CON (High-Fat Diet Control), SD-CON (Standard-Diet Control), SD-AA (Standard-Diet + Amino-acid Mixture), HF-AA (High-Fat Diet + Amino-acid Mixture) treatments were gavaged with glucose. Blood was collected from the tail vein before gavage and 15, 30, 60, and 120 minutes after gavage. Values are mean  $\pm$  SE. \* $p$ <0.05 HF-CON vs. all other treatments. \*\* $p$ <0.05 HF-CON vs. SD-CON, SD-AA. ‡ $p$ <0.05 HF-AA vs. SD-CON, SD-AA.

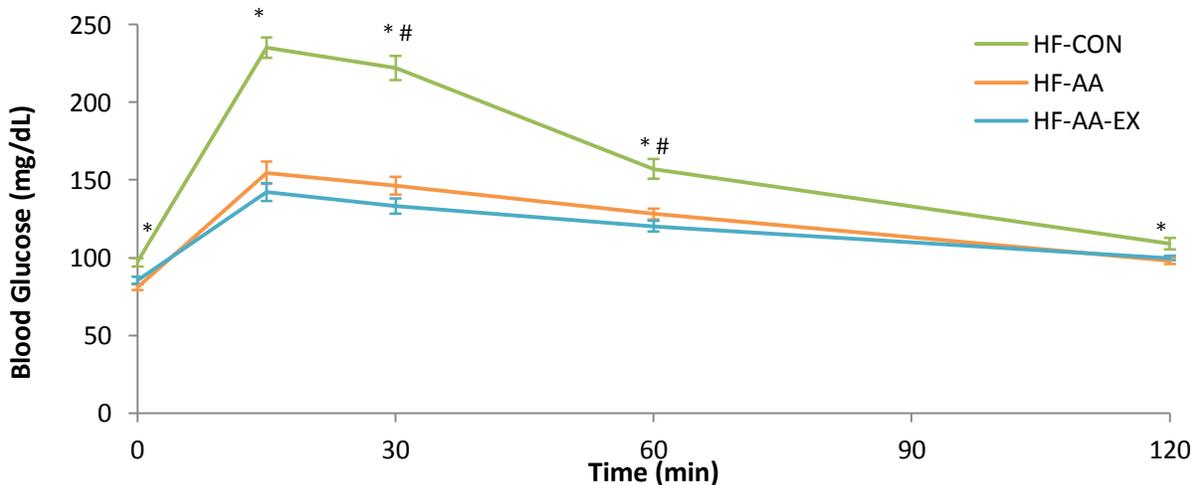


**Fig. 5. Plasma insulin during OGTT at 4 weeks.** Sprague-Dawley rats in HF-CON (High-Fat Diet Control), SD-CON (Standard-Diet Control), SD-AA (Standard-Diet + Amino-acid Mixture), HF-AA (High-Fat Diet + Amino-acid Mixture) treatments were gavaged with glucose. Blood was collected from the tail vein before gavage and 15, 30, 60, and 120 minutes after gavage. Values are mean  $\pm$  SE. \* $p$ <0.05 HF-CON vs. all other treatments. \*\* $p$ <0.05 HF-CON vs. SD-CON, SD-AA. ‡ $p$ <0.05 HF-AA vs. all other treatments. ‡‡ $p$ <0.05 HF-AA vs. SD-CON, SD-AA.

Further analysis can be done by comparing the high-fat diet groups (High-Fat Diet Control–HF-CON, High-Fat Diet and Amino-Acid Mixture–HF-AA, High-Fat Diet and Amino-Acid Mixture plus Exercise Treatment–HF-AA-EX). After two weeks, the OGTT (Fig. 6) showed a significantly higher blood glucose level for the HF-CON group compared with the treatment groups, but no difference between the HF-AA and HF-AA-EX. In contrast, in the four-week OGTT (Fig 7), the HF-AA treatment had a significantly higher blood glucose level at 30 and 60 minutes compared to HF-AA-EX. The HF-CON remained significantly elevated above the treatment groups in the 4-week OGTT, and also reached a much higher peak at 15 and 30 minutes compared with the OGTT at 2 weeks.

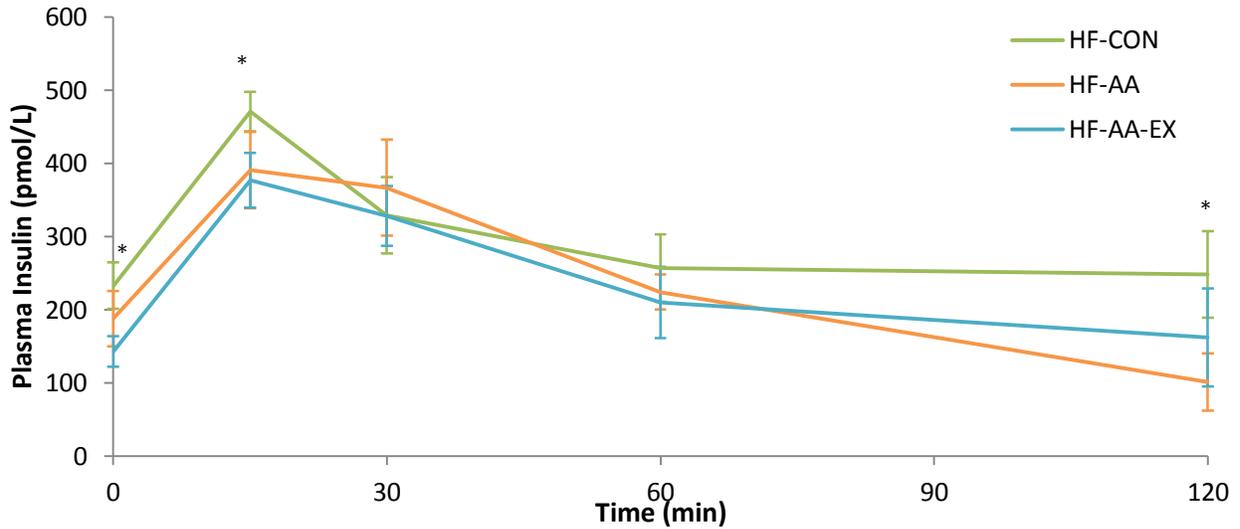


**Fig. 6. Oral Glucose Tolerance Test (OGTT) at 2 weeks.** Sprague-Dawley rats in HF-CON (High-Fat Diet Control), HF-AA (High-Fat Diet + Amino Acid Mixture), HF-AA-EX (High-Fat Diet + Amino-acid Mixture + Exercise Treatment) treatments were gavaged with glucose. Blood was collected from the tail vein before gavage and 15, 30, 60, and 120 minutes after gavage. Values are mean  $\pm$  SE. \* $p$ <0.05 HF-CON vs. all other treatments.

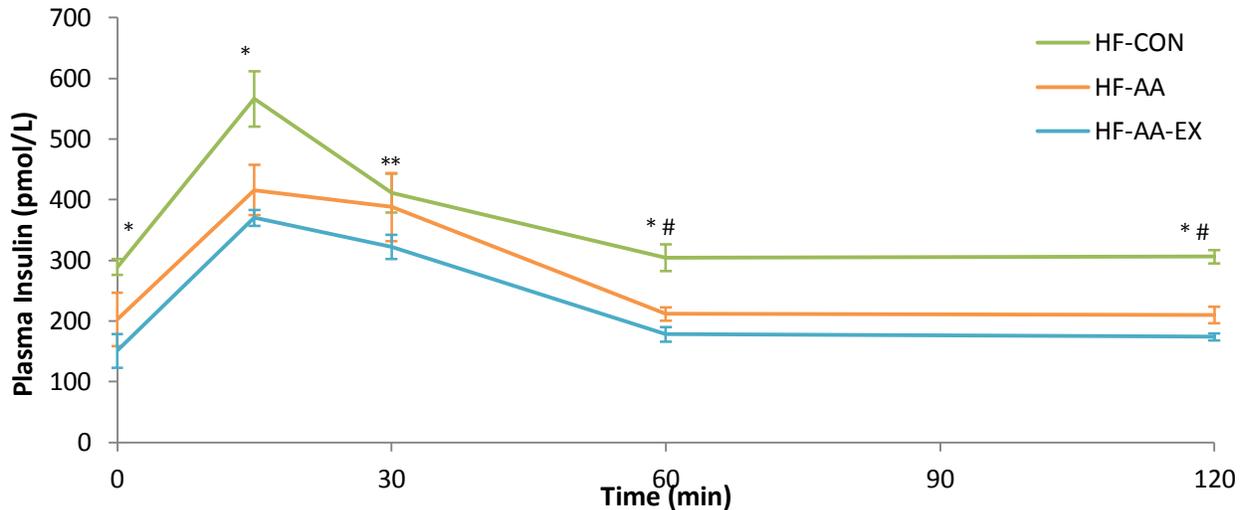


**Fig. 7. Oral Glucose Tolerance Test (OGTT) at 4 weeks.** Sprague-Dawley rats in HF-CON (High-Fat Diet Control), HF-AA (High-Fat Diet + Amino Acid Mixture), HF-AA-EX (High-Fat Diet + Amino-acid Mixture + Exercise Treatment) treatments were gavaged with glucose. Blood was collected from the tail vein before gavage and 15, 30, 60, and 120 minutes after gavage. Values are mean  $\pm$  SE. \* $p$ <0.05 HF-CON vs. all other treatments. # $p$ <0.05 HF-AA-EX vs. HF-AA.

During the 2-week OGTT, plasma insulin levels were significantly higher in HF-CON compared with HF-AA and HF-AA-EX at 0, 15, and 120 minutes. No other statistically significant differences were observed. In the 4-week OGTT, HF-CON plasma insulin was significantly higher than both treatment groups at all time points except at 30 minutes, when HF-CON was only significantly higher than HF-AA-EX. At 60 and 120 minutes, HF-AA-EX plasma insulin was significantly lower than HF-AA.



**Fig. 8. Plasma insulin during OGTT at 2 weeks.** Sprague-Dawley rats in HF-CON (High-Fat Diet Control), HF-AA (High-Fat Diet + Amino Acid Mixture), HF-AA-EX (High-Fat Diet + Amino-acid Mixture + Exercise Treatment) treatments were gavaged with glucose. Blood was collected from the tail vein before gavage and 15, 30, 60, and 120 minutes after gavage. Values are mean  $\pm$  SE. \* $p$ <0.05 HF-CON vs. all other treatments.



**Fig. 9. Plasma insulin during OGTT at 4 weeks.** Sprague-Dawley rats in HF-CON (High-Fat Diet Control), HF-AA (High-Fat Diet + Amino Acid Mixture), HF-AA-EX (High-Fat Diet + Amino-acid Mixture + Exercise Treatment) treatments were gavaged with glucose. Blood was collected from the tail vein before gavage and 15, 30, 60, and 120 minutes after gavage. Values are mean  $\pm$  SE. \* $p$ <0.05 HF-CON vs. all other treatments. \*\* $p$ <0.05 HF-CON vs. HF-AA-EX. # $p$ <0.05 HF-AA-EX vs. HF-AA.

#### 4. Discussion

A high-fat diet has been shown to induce insulin resistance in rats, resulting in increased plasma insulin and hyperglycemia (Storlein et al., 1986). Research has shown that exercise prevents high-fat diet induced insulin resistance (Friedrichsen et al., 2012; Liao & Xu, 2011; Sano et al., 2012), and also increases glucose transport rate (Jung & Kang, 2010). Research has also confirmed the glucose-lowering effects of branched chain amino acids in rats fed a standard diet (Bernard et al., 2011; Kleinert et al., 2011). No known research has examined the effects of amino acids, and amino acids coupled with exercise, in rats fed a high-fat diet. Therefore the purpose of this investigation was to analyze the effects of amino-acids and exercise on glucose tolerance and plasma insulin levels in rats fed either a standard diet or a high-fat diet.

The first part of this analysis looked at the effects of a pre-established amino acid mixture with increased isoleucine concentration. Within the first two weeks the effects of the high-fat diet control treatment already are evident as insulin resistance begins to develop. This is evidenced by the high blood glucose levels and elevated plasma insulin, suggesting that insulin is unable to be utilized to bring glucose into the skeletal muscle cells. After four weeks, insulin resistance has developed further, as blood glucose levels are significantly higher than in rats fed a standard diet. The increased blood glucose is coupled with significantly high plasma insulin levels, further evidencing that insulin is unable to be utilized. This is consistent with previous research showing that a high-fat diet results in the inactivation of IRS-1-PI3K activity (Liao & Xu, 2011; Tremblay et al., 2001) and thus the inability to use insulin to bring glucose into the cell.

Interestingly, at two weeks, the amino-acid mixture coupled with the high-fat diet resulted in the lowest blood glucose levels despite plasma insulin levels above the standard diet treatments. After four weeks, the high-fat diet plus amino-acid treatment group continued to have low levels of blood glucose, at a level similar to the standard diet amino-acid treatment group despite increased plasma insulin. Since the levels of the two amino-acid treatments have similar levels of blood glucose, this suggests that amino-acids may help regulate blood glucose levels effectively enough to mitigate the effects of insulin resistance resulting from the high-fat diet. Nevertheless, it appears that signs of insulin resistance likely still develop in these rats fed a high-fat diet due to the elevated insulin levels compared with treatment groups fed a standard-diet. However, plasma insulin is significantly lower for the HF-AA treatment group compared with the HF-CON group at all time points, except at 30 minutes during the four weeks OGTT, suggesting that amino-acids may play some role in alleviating the development of insulin resistance by helping cells utilize insulin for glucose transport. The data supports the research suggesting that amino-acids work through at least a partially separate pathway from the insulin pathway (Kleinert et al., 2011) since the amino-acids are able to effectively lower blood glucose despite high plasma insulin. Further research looking at GLUT-4 content as well as intracellular signaling proteins is necessary to help clarify how amino-acids affect insulin resistance. Our study confirmed that in rats fed a standard-diet, an amino-acid treatment is effective at lowering blood glucose without an increase in plasma insulin.

The second part of this analysis compared the treatment groups of rats fed a high-fat diet. Both the amino-acid mixture and exercise treatment were effective in lowering blood glucose levels despite the administration of a high-fat diet when compared with the

high-fat diet control group. Research supports exercise as an effective treatment for preventing and alleviating insulin resistance (Friedrichsen et al., 2012; Liao & Xu, 2011; Sano et al., 2012). Our data confirmed these findings since the exercise treatment lowered blood glucose without high plasma insulin levels. However, our study coupled the exercise treatment with the amino-acid treatment to look for any additive effects. After four weeks, the amino-acid plus exercise treatment group showed significantly lower blood glucose levels at 30 minutes and 1 hour compared with just amino-acids alone, suggesting that exercise provides an additive effect. This confirms that amino-acids and exercise work through separate pathways. Since the significant difference only appeared after four weeks, but not at two, it is possible that the exercise treatment takes time to create changes in cell glucose utilization and/or GLUT-4 translocation. Further research examining total GLUT-4 content will allow us to see if increased GLUT-4 explains the difference between the amino-acid treatment and amino-acid plus exercise treatment.

Prior to this study, research found that amino-acids were effective at lowering blood glucose, suggesting a role for an amino-acid mixture as a non-invasive treatment for improving control of glucose levels. The implications of this study suggest that amino-acids may have an even farther reaching efficacy in controlling blood glucose in the presence of insulin resistance. Amino-acids coupled with exercise may result in increased benefits of glucose tolerance. Further research, examining all possible control and treatment groups, will provide a full picture of just how effective these treatments are in improving blood glucose levels in conditions with, or without, insulin resistance.

In conclusion, this study found that amino-acids are effective at lowering blood glucose in rats with normal glucose tolerance as well as rats made insulin resistant through a high-fat diet. Additionally, an exercise treatment provides an additive effect when coupled with amino-acids in lowering blood glucose levels. Plasma insulin levels are increased in all groups fed a high-fat diet compared with those fed a standard diet, but insulin is lower for amino-acid supplemented groups versus the control, and still lower for the amino-acid plus exercise treatment group. Further research examining all treatment groups, total GLUT-4 content of all groups, as well as various intracellular proteins is necessary to provide a full picture of the physiological effects that amino-acids, diet, and exercise have on skeletal muscle glucose tolerance.

## **Acknowledgments**

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