The Effect Of Butyrate Feeding On Skeletal Muscle And Adipose Tissue Glucose Uptake

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Master of Science in Biology

by
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Accepted by the faculty of the College of Science and Technology, Morehead State University, in partial fulfillment of the requirements for the Master of Science degree.

[Signatures]

Director of Thesis

Master's Committee

Date 5/31/02
There is an intricate relationship between free fatty acids (FFA), insulin receptor/substrate interaction, and GLUT4 translocation to the plasma membrane in skeletal muscle and adipose tissue. Previous literature has shown that there is a direct relationship between high plasma FFA levels and impaired insulin-stimulated glucose transport (Hansen, et al., 1998). Over time this relationship induces obesity and Type 2 Diabetes. Human and animal research has been used to understand the influences of long-chain FFA, but few have studied short-chain FFA under acute exercise stimulation. Therefore, the goal of this research was to study the effects of butyrate on skeletal muscle and adipose tissue glucose uptake with or without exercise on untrained mice.

BALB/c mice were separated into saline, glucose, and butyrate groups and given an intraperitoneal injection of (\(^{14}\text{C}\)) 2-deoxyglucose (2-DG) plus caloric source before sacrifice. Three different skeletal muscles from the mouse hindlimb and perigonadal adipose tissue were dissected, prepared, and scintillation counts were determined. Forced-swim tests were also performed to evaluate the effects of butyrate and acute exercise on 2-DG uptake. Under acute exercise, skeletal muscle showed a significant decrease in 2-DG uptake under butyrate conditions: 54%
(soleus, 80% SO fiber composition), 20% (extensor digitorum longus, 76% FG fiber composition), 42% (gastrocnemius, 6-12% SO fiber composition) compared to saline-treated controls.

Glucose and exercise exhibited a quantitatively additive effect in gastrocnemius muscle when compared to saline exercise and glucose sedentary groups. Finally, exercise demonstrated that muscle fiber type proved influential in 2-DG uptake. These results further reinforce the importance of caloric source and exercise in the regulation of glucose uptake.

Accepted By: 

Chair

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Introduction

Glucose, one of the primary energy substrates in metabolism, enters various mammalian cells through glucose (GLUT) transporters. There are seven known transporters (see Table 1) in various tissues (Hargreaves, 1999). These transporters follow typical saturation kinetics and rely on concentration gradients to move across the plasma membrane. However, some of the transporters do not reside within the plasma membrane, but are contained within membranes of the cytoplasmic vesicles that fuse or invaginate depending on the stimulus. There are numerous stimuli that may induce these exo- or endocytotic responses.

Table 1: Function and tissue distribution of GLUT family transporters (taken from Hargreaves, Biochemistry of Exercise X, 1999).

<table>
<thead>
<tr>
<th>Isoform</th>
<th>Tissue Distribution</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLUT1</td>
<td>Ubiquitous, High expression in brain endothelial cells and human erythrocytes</td>
<td>Basal glucose transport Located in plasma membrane Low Km (2-3 mM)</td>
</tr>
<tr>
<td>GLUT2</td>
<td>Liver, kidney and small epithelial cells, β-cells of pancreas</td>
<td>High Km (15-20 mM) Glucose transport in and out of hepatocytes and β-cells</td>
</tr>
<tr>
<td>GLUT3</td>
<td>Neurons, placenta</td>
<td>Low Km (2-3 mM) Transport into CNS</td>
</tr>
<tr>
<td>GLUT4</td>
<td>Skeletal muscle, brown and white adipose tissue, heart</td>
<td>Mediates insulin and contraction regulated glucose transport Km (5-6 mM)</td>
</tr>
<tr>
<td>GLUT5</td>
<td>Small intestine, kidney, skeletal muscle, human adipocytes</td>
<td>Fructose transporter</td>
</tr>
<tr>
<td>GLUT7</td>
<td>Liver</td>
<td>Part of the glucose-6-phosphatase complex in endoplasmic reticulum</td>
</tr>
</tbody>
</table>
One important effector of glucose transport is insulin. Insulin, composed of 51 amino acids, has an alpha- and beta-chain with two interchain disulfide bridges and one intrachain disulfide bridge. Elevated blood glucose concentrations cause insulin to be released from the β-cells in the pancreas into the bloodstream. Several tissues (neural, cardiac, skeletal, etc.) express insulin receptors that bind to insulin. Insulin binding activates intracellular, tyrosine kinase domains on the β-subunit of the insulin receptor. This results in autophosphorylation of a number of tyrosines on these domains and subsequent activation of various effectors of insulin action including insulin receptor substrate-1 (IRS-1). Subsequently, the p85 subunit of phosphatidylinositol-3 kinase (PI3K) becomes active. One component of insulin action on GLUT4 translocation is through PI3K. This branch of the insulin signaling cascade activates GLUT4 vesicles that will then move toward the plasma membrane (and transverse tubule membranes in skeletal muscle) as indicated in Figure 1 (Hansen, et al., 1996).

Figure 1: Insulin signaling cascade in skeletal muscle (taken from Hansen, et al., 1996)
Upon docking with the plasma membrane, the vesicle becomes fused with the plasma membrane exposing the transporters to the extracellular surface of the cell, facilitating diffusion of glucose into the cell. Once glucose equilibrium is reached, clathrin coats the membrane to allow for vesicle internalization. In addition to PI3K activation, other various intracellular effectors of insulin action have been implicated in GLUT4 translocation, such as protein kinase B/Akt (Turinsky, J. et al., 1999), phospholipase C (Griffin, M., et al., 1999, Epps-Fung, M., et al., 1997), and phospholipase D (Emoto, M., et al., 2000).

Currently, skeletal muscles possess three, characterized GLUT transporters. The first discovered GLUT transporter was GLUT1, which constitutively resides within the plasma membrane. Its function is basal glucose transport and is not responsive to insulin levels. Another transporter found constitutively within the membrane is GLUT5, which is responsible for fructose transport. Lastly, one of the most important transporters in skeletal muscle is GLUT4, which is contained within vesicles in the cytosol of muscle cells. GLUT4 transporters are responsible for 80% of glucose disposal in skeletal muscle under 3-10 mM insulin-stimulated conditions (Holloszy, et al., 1998). Meckler (2001) notes that 90% of insulin-stimulated GLUT4 translocates to the transverse-tubule surface allowing quick penetration of glucose to the core of the muscle fiber.

Skeletal muscle can be divided into three, different muscle fiber types: slow-oxidative (SO), fast-oxidative glycolytic (FOG), fast-glycolytic (FG) and each have various characteristics as shown in Table 2 (Sherwood, 1997). FG fibers have
higher myosin-ATPase activity than SO fibers allowing greater myosin-actin cross-bridge formation to generate muscle tension but in less time. One reason for reduced duration of sustained muscle contraction stems from the fact that FG fibers essentially derive ATP from lactate fermentation and the working muscle fatigues in response to increased lactate levels. SO fibers, on the other hand, have abundant

Table 2: Characteristics of skeletal muscle fibers (taken from Sherwood, Human Physiology, from Cells to Systems, 1997.)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Slow-oxidative (SO)</th>
<th>Fast-oxidative glycolytic (FOG)</th>
<th>Fast-glycolytic (FG)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myosin-ATPase activity</td>
<td>Low</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Speed of Contraction</td>
<td>Slow</td>
<td>Fast</td>
<td>Fast</td>
</tr>
<tr>
<td>Presence of Mitochondria</td>
<td>Many</td>
<td>Many</td>
<td>Few</td>
</tr>
<tr>
<td>Capillaries</td>
<td>Many</td>
<td>Many</td>
<td>Few</td>
</tr>
<tr>
<td>Myoglobin Content</td>
<td>High</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Color of Fiber</td>
<td>Red</td>
<td>Red</td>
<td>White</td>
</tr>
<tr>
<td>Resistance to Fatigue</td>
<td>High</td>
<td>Intermediate</td>
<td>Low</td>
</tr>
<tr>
<td>Glycogen Storage</td>
<td>Low</td>
<td>Intermediate</td>
<td>High</td>
</tr>
<tr>
<td>Oxidative-Phosphorylation</td>
<td>High</td>
<td>High</td>
<td>Low</td>
</tr>
</tbody>
</table>
mitochondria which produce ATP by oxidative phosphorylation, have much less reliance on lactate fermentation and are more resistant to fatigue. SO fibers can produce higher amounts of ATP by utilizing proteins, carbohydrates, and fatty acids as energy sources, whereas FG fibers are primarily dependent on glucose and glycogen. FOG fibers play an intermediate role between these two extremes by resembling FG fibers in myosin-ATPase activity and SO fibers in number of mitochondria and myoglobin content (Sherwood, 1997). FOG fibers are adapted to the needs of the organism and constitute a majority of the total muscle content in mammals (Hargreaves, 1999). Further similarities and differences between these different fibers types are listed in Table 2 (Sherwood, 1997).

Another distinguishing characteristic is the maximum volume of oxygen uptake in a one-minute time interval (VO₂ max). The VO₂ max quantitates the intensity of exercise which plays a significant role in muscle performance (Hawley, et al., 1997). A high VO₂ max (80-90%) indicates a high-intensity workout and will result in early FG fiber fatigue. A low VO₂ max (20-50%) indicates a low-intensity workout and will through training, build endurance for FG fiber types. Some exercises, such as swimming, can be classified as a moderate-intensity workout (with percentages between 50-80%) (Hargreaves, 1999). Unlike FG fibers, SO fibers (as found in diaphragm) muscle adapt and are much more resistant to fatigue upon intense exercise. The diaphragm is partially responsible for both inspiration and expiration of air and is continually influenced in a positive fashion to exercise so that oxygen can be supplied and carbon dioxide can be extracted (Sherwood, 1997).
In rodents, there are different muscles within the hindlimb and each have a variable amount of muscle fiber types (refer to Figure Two). The soleus has an 80% composition of SO fibers (Hargreaves, 1999). It lies underneath the gastrocnemius muscle and is used primarily to hold the animal up against the force of gravity. The gastrocnemius muscle, on the other hand, is predominantly composed of FG and FOG fibers with only 6-12% of the fibers being slow-oxidative (Hargreaves, 1999). The gastrocnemius is located in the lower leg and is used to generate thrust and motion. The extensor digitorum longus (EDL) has a 76% composition of FG fibers and is located under the anterior tibialis in the front of the lower leg (Hargreaves, 1999). Similar to the gastrocnemius, it too is responsible for locomotive ability.

![Figure 2: Anatomical diagram of hindlimb muscles in the guinea pig (taken from Cooper, 1975)](image-url)
In addition to insulin stimulation, glucose transport is also stimulated by muscle contraction. Research has shown that insulin and contraction stimulation of glucose transport in skeletal muscle appears to be mediated through two, distinct pathways (Lund, et al, 1995). *In vitro* studies have shown that wortmannin, a potent inhibitor of PI3K, caused a decrease in insulin stimulation but had no effect on contraction-stimulated GLUT4 translocation. Contraction elicits calcium release from the sarcoplasmic reticulum, but calcium levels rise and fall within a fraction of a second; yet such a transient stimulus is sufficient to activate GLUT4 translocation to the membrane (Hansen et al., 1996). Therefore, an intracellular mechanism that is calcium-sensitive must be involved in order for GLUT4 translocation to occur. Research has speculated on a number of different proteins and autocrine signals, including mitogen-activated protein kinase (MAPK), nitric oxide (NO), and adenosine (Hayashi, et al., 1997). However, the current mechanism by which contraction mediates GLUT4 translocation in skeletal muscle has not been elucidated.

Studies have also shown that insulin and exercise exhibit additive effects in glucose transport. Gao and others show a 113% increase in glucose transport using insulin infusion followed by contraction *in situ* (Gao, et al., 1994). Other *in vitro* studies indicate that contraction and insulin combined, stimulated a four-fold increase in glucose transport compared to controls (Lund et al., 1995). Such a wide range in results could be indicative on the methodology used in these respective experiments.
Despite these two different pathways, fatty acid concentrations can affect both. It is known that individuals on long-term fat-enriched diets develop insulin resistance and β-cell failure in the pancreas, contributing to Type 2 Diabetes. In rodents, researchers have shown that a high fat diet for a period of 8 weeks showed a significant decrease in total GLUT4 levels on the cell surface when compared to the controls (Hansen et al., 1998). These studies were performed in vitro and decreases were found for both insulin- and contraction-stimulated skeletal muscle. However, it was not stated in their methodology, what type of fat (saturated vs. unsaturated) or chain length (long vs. short) was used, only that 50% of the animal’s caloric intake came from fat. Furthermore, no studies were performed on adipose tissue, which is responsible for fat degradation and synthesis.

Adipose cells are dispersed within loose connective tissue and contain droplets of fat and glycogen inclusion bodies that serve as an energy reserve for the starvation state. Adipose tissue is responsive to insulin which mediates its effect by increasing GLUT4 recruitment to the plasma membrane (Sherwood, 1997). Insulin inhibits lipolysis and stimulates lipogenesis (Sherwood, 1997). Lipolysis allows the release of free fatty acids (FFA) to tissues that can readily use them.

Randle and others (1965) first proposed the glucose-fatty acid cycle as a way of understanding how FFA inhibited glucose uptake in skeletal muscle. Long-chain acyl-CoAs enter the mitochondria through the carnitine palmitoyl transferase (CPT) system. As FFA enter mitochondria, an accumulation of acetyl-CoAs and citrate result in inhibition of pyruvate dehydrogenase and phosphofructokinase-1
respectively, thus slowing glycolysis. Acetyl-CoA enters the citric acid cycle (TCA) and generates NADH/FADH₂, which enter the electron transport chain accounting for the majority of ATP production. Inhibition of glycolysis results in increased levels of glucose-6-phosphate (G6P), which inhibits hexokinase and consequently glucose import. It has not been determined however, if this is the only mechanism by which FFA inhibit glucose uptake or transport during contraction.

The goal of these experiments is to look at the effects of butyrate and exercise on skeletal muscle and adipose tissue glucose uptake. By using mice as a model of study, radiolabelled glucose can be injected intraperitoneally in combination with different caloric sources (Ohira, et al., 1994). These experiments are the first to focus on a specific fatty acid and look at acute regulation of glucose transport in various tissues.

The hypothesis for my experiments is that the combination of butyrate and exercise will impair glucose uptake in skeletal muscle and enhance glucose uptake in adipose tissue.
Materials & Methods

Twenty-two male, 5-7 week-old BALB/c mice were purchased from Harlan Sprague Dawley Inc., and were housed in the Lappin Hall Animal Care Facility. Animals were housed three per cage under a twelve hour light/dark cycle and fed Purina Lab chow and water *ad libitum*. The animals underwent a diet training program that consisted of a twelve hour fast, one hour of 4% liquid sucrose, and an eleven hour chow and water regiment. This diet training lasted for 2 weeks and animals were randomly selected for sedentary or swim exercise based on three, different caloric sources (none, glucose, or butyrate). (Therefore, six different treatment groups were created: saline-sedentary, saline-exercise, glucose-sedentary, glucose-exercise, butyrate-sedentary, and butyrate-exercise). On the day before experiments took place, animals were fasted for a period of 24 hours.

**Blood Glucose Determinations**

Blood glucose measurements (Sigma Diagnostics, Glucose Trinder Reagent) were taken before injections and after cervical dislocation. Ten microliters of blood were placed with 1 ml of glucose trinder reagent at room temperature. Five microliters of deionized water was added to each eppendorf tube and allowed to incubate for 18 minutes. Each sample was placed in a 1 ml plastic cuvette and read at 505 nm in a spectrophotometer. A standard was made placing 1 ml of glucose trinder reagent with 5 µl of deionized water after a 18 minute incubation at room temperature. Blood glucose levels were calculated using the following formula:

Serum Glucose Concentration (mg/dl) = \frac{\text{Abs. of sample}}{\text{Abs. of standard}} \times 100
**Intraperitoneal Injections of (¹⁴C) 2-Deoxyglucose**

Mice were injected intraperitoneally (1 cc syringe with a 27-gauge needle) with 10 µCi of (¹⁴C) 2-DG (specific activity = 51 mCi/mmol) at a dose of 20 µl per 480 µl of caloric source. One of the caloric sources, butyrate, is a four-carbon saturated fat that is found in butter and cheese. It yields 29 ATP through 1 FADH₂, 1 NADH, and 2 acetyl-CoA (2.5 ATP = 1 NADH and 1.5 ATP = 1 FADH₂).

However, 2 ATP equivalents are consumed through coenzyme A activation in the cytosol giving a net of 27 ATP. When compared with glucose, the ATP potential for each carbon is seven for butyrate and only six for glucose. The caloric potential for fats is also much higher than carbohydrates, 9 kcal/g and 4 kcal/g, respectively (Randall, et al., 1997). ATP potential was calculated for saline, glucose, and butyrate. ATP generation for each caloric source was 0, 36, and 27 ATP, respectively. Therefore, butyrate concentration was set at 1 M, pH = 7 and 27/36 of the butyrate concentration totaled 0.75 M for the concentration of glucose. This was to insure that equimolar concentrations of ATP were generated. Deoxyglucose is a non-metabolizable substrate due to the presence of a hydrogen at the 2-carbon (See Figure 3 for comparison between 2-DG and glucose). Therefore, 2-DG can become phosphorylated by hexokinase but cannot proceed further in glycolysis.

![Beta-D-glucose](image1.png)  ![2-deoxyglucose (2-DG)](image2.png)

**Figure 3**: Structural differences between 2-DG and β-D-glucose.
Furthermore, 2-DG does not elicit an insulin response whereas glucose will allow for insulin secretion.

Isolation of soleus, extensor digitorum longus (EDL) muscles and other tissues

After cervical dislocation, an ankle bracelet was cut and skin removed to expose the leg muscles. Scissors were placed under the Achilles tendon and opened to push back connective tissue. The scissors were then placed next to the calcaneous bone and the Achilles tendon was cut. The clipped tendon was grasped with a hemostat and pulled downward exposing the soleus muscle. This stripping action was continued until the upper tendon was exposed. The upper and lower tendons of the soleus muscle were cut, releasing the muscle, and a scapal was used to remove excess tendon and connective tissue.

For the EDL muscle, the tendon attached to the anterior tibialis muscle was cut with a scapal. The tendon “popped up” allowing the removal of the anterior tibialis. This exposed the EDL and upper tendon attached to the knee. The upper tendon was cut with a scapal and fine forceps were used to remove the muscle (this should be performed in the downward motion, too). The lower tendon was exposed and cut with straight scissors.

The other eight tissues consisted of the gastrocnemius, anterior tibialis, liver, large intestine, heart, testes, kidneys and adipose tissue. The gastrocnemius and anterior tibialis were dissected out after soleus and EDL removal. Adipose tissue was extracted from the perigonadal region and coupled with extraction of testes. The next sample tissues removed were the large intestine, liver, kidneys, and
heart. A section of large intestine and liver were studied while kidneys and heart were dissected out whole.

Tissues were rinsed with Delbecco’s phosphate-buffered saline and blotted dry on filter paper. Tissues were then weighed on an analytical balance and placed into individual eppendorf tubes. Tubes were housed in the -70°C freezer overnight. The next day, samples were taken out of the freezer and placed in a dry, dark area for a period of 12 hours. This was to preserve the integrity of the radiolabel and to allow the plastic tubes a chance to reach room temperature. Next, one milliliter of distilled water was added to each tube and placed in a boiling water bath for 10 minutes. Then tubes were placed in an ice bath for 10 minutes. Finally, tubes were centrifuged at 14K rpm for 15 minutes and a 200 µl aliquot was combined with 6.5 mls of scintillation cocktail. Counts were detected by a Beckman LS 1801 counter for a period of ten minutes and expressed in counts per minute (CPM).

**Exercise protocol**

Mice on exercise regimes were given a 15 minute swim in a Pyrex© tub maintained at six inches in depth and heated to 35°C. This was followed by a 45 minute rest for treatment groups. Experimental design is depicted in Figure 4.

![Figure 4: Diagram of experimental design for intraperitoneal injections](image-url)
**Statistical analysis**

Testes showed the least variability among the treatment groups (< 10%) for the ten tissues collected. Therefore, data collected from the testes was used to compare differences among the other tissues. Data was collected and interpreted as CPM/g wet tissue weight. When dividing the uptake of glucose in one tissue against that of the testes, the answer yields a dimensionless number. Therefore, the units are arbitrary.

Statistical analysis was calculated using Sigma Stat and Plot programs (Jandel Scientific, Inc.) One-way analysis of variance (ANOVA) was performed among the three different diets and t-tests were used to test differences among the exercise and sedentary groups. Significance was reported at p< 0.05.
Results

**Blood glucose determinations**

Blood glucose concentrations were determined before and after 2-DG injections as shown in Figure 5. Blood glucose levels in saline-treated mice were fairly constant before and after 2-DG injections in both exercised and sedentary conditions. Glucose-treated exercised mice had significant increases in blood glucose when compared to saline-treated and butyrate-treated mice. There was also a significant difference before and after treatments for the glucose exercise group. Butyrate-exercise animals had a slight elevation in blood glucose before the treatment, but decreased after sacrifice.

![Blood glucose determinations](image)

**Figure 5**: Blood glucose determinations before and after treatments in saline, glucose, and butyrate-treated mice. Sedentary and exercise groups are indicated as "sed." and "exe." Asterisks indicate significance between before and after 2-DG injections in glucose exercise mice. A comparison was made between the after glucose exercise treatment group and other exercise treatments. The pound symbol (#) indicates significance for this comparison. Significance is reported at p< 0.05 (n = 19).
2-DG uptake in various tissues

The raw 2-DG uptake data is supplied in the Appendix I. These data were normalized to the 2-DG value of testis so as to provide an internal standard by which to control for differences in glucose availability to all tissues from mouse to mouse. These normalized values are listed for nine tissues in Table 3. Testis uptake was not reported, since its ratio would be recorded as one.

Table 3: 2-DG uptake for the nine treatment groups. All data is expressed as ratios between respective tissue and testis 2-DG glucose uptake. The data is expressed as mean ± SEM. Significance is reported at p < 0.05. *If significance is reported, it may be for one or two of the diet/exercise groups. Abbreviations for saline, glucose, and butyrate groups are indicated as S, G, and B, respectively. The blackened circle (•) indicates not enough replicates were recorded to perform statistical analysis. Raw data is reported in Appendix One.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Nonexercise</th>
<th>Exercise</th>
<th>Saline</th>
<th>Glucose</th>
<th>Butyrate</th>
<th>Signific. of Diet* (p&lt;0.05)</th>
<th>Signific. of Exercise* (p&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soleus</td>
<td></td>
<td></td>
<td>7.44 ± 0.41</td>
<td>11.38 ± 0.47</td>
<td>3.99 ± 0.55</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Nonexercise</td>
<td>3.39 ± 0.39</td>
<td>4.64 ± 0.42</td>
<td>0.63 ± 0.15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>0.28 ± 0.27</td>
<td>0.807 ± 0.48</td>
<td>0.431 ± 0.33</td>
<td></td>
<td>no</td>
<td></td>
<td>yes, B</td>
</tr>
<tr>
<td>Exercise</td>
<td>0.55 ± 0.25</td>
<td>1.07 ± 0.94</td>
<td>3.71 ± 1.60</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>0.781 ± 0.71</td>
<td>0.807 ± 0.48</td>
<td>0.904 ± 0.80</td>
<td></td>
<td>no</td>
<td></td>
<td>yes, S &amp; G</td>
</tr>
<tr>
<td>Nonexercise</td>
<td>0.486 ± 0.2</td>
<td>3.15 ± 1.61</td>
<td>2.74 ± 0.60</td>
<td></td>
<td>no</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastrocnemius</td>
<td>0.717 ± 0.14</td>
<td>1.29 ± 0.04</td>
<td>0.599 ± 0.12</td>
<td>yes, G</td>
<td></td>
<td>yes, G S vs B</td>
<td></td>
</tr>
<tr>
<td>Exercise</td>
<td>0.974 ± 0.14</td>
<td>2.11 ± 0.09</td>
<td>0.406 ± 0.10</td>
<td></td>
<td>yes, G</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>EDL*</td>
<td></td>
<td></td>
<td>1.08 ± 0.22</td>
<td>7.49 ± 0.87</td>
<td>2.73 ± 0.42</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Exercise</td>
<td></td>
<td></td>
<td>13.3</td>
<td>4.12 ± 0.22</td>
<td>7.49 ± 0.87</td>
<td>2.73 ± 0.42</td>
<td>yes, S G</td>
</tr>
<tr>
<td>Anterior Tib.</td>
<td>0.87 ± 0.04</td>
<td>1.80 ± 1.27</td>
<td>2.77 ± 0.49</td>
<td></td>
<td>no</td>
<td></td>
<td>yes</td>
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<tr>
<td>Exercise</td>
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The effects of exercise on 2-DG uptake

Soleus muscle results: Whole soleus muscle was extracted and the results are depicted in Figure 6. Exercise produced a significant decrease in 2-DG uptake in soleus muscle under saline, glucose, and butyrate conditions. This characteristic was not observed for the other muscle groups; however, the soleus muscle was the only muscle predominantly composed of slow-oxidative fibers. Exact values and standard error measurements (SEM) are indicated in Table 3.

Figure 6: 2-DG uptake in soleus muscle for the six treatment groups. All data is expressed as mean ± SEM. Asterisks above the bars are significant in comparison of sedentary versus exercise groups. Significance is at p < 0.05 (n = 10). Exact values and SEM measurements are reported in Table 3. Data is normalized to testis uptake.
**Gastrocnemius muscle results:** Whole gastrocnemius muscle was extracted and the results are depicted in Figure 7. In glucose-treated animals, exercise significantly increased 2-DG uptake. While exercise increased the level of 2-DG uptake in saline-treated controls and decreased uptake in butyrate-treated animals, those results were not statistically significant. The glucose-exercise group increase in 2-DG uptake was 107% of the sum of the increase seen in glucose-sedentary and saline-exercise. Butyrate-treated mice did not show a significant difference between sedentary and exercise groups.

![Figure 7: 2-DG uptake in gastrocnemius muscle for the six treatment groups. All data is expressed as mean ± SEM. Asterisks above the bars are significant in comparison of sedentary versus exercise groups. Significance is at p <0.05 (n = 10). Exact measurements and SEM values are reported in Table 3. Data is normalized to testis uptake.](image)
**Adipose tissue results:** Perigonadal adipose tissue was extracted and the results are depicted in Figure 8. Exercise produced a significant decrease in adipose tissue 2-DG uptake in saline-treated mice.

![Figure 8: 2-DG uptake in adipose tissue for the six treatment groups. All data is expressed as mean ± SEM. Asterisks above the bars are significant in comparison of sedentary versus exercise groups. Significance is at p < 0.05 (n = 10). Exact measurements and SEM values are reported in Table 3. Data is normalized to testis uptake.](image)
Comparison of caloric source and its effect on 2-DG uptake in skeletal muscle and adipose tissue.

**Soleus muscle results:** As shown in Figure 9, glucose treatment increased 2-DG uptake for the sedentary state in soleus muscle. Butyrate significantly decreased 2-DG uptake. Exercised mice showed the same significant trends, but again a decrease in 2-DG uptake is evident between sedentary and exercise groups.

![Figure 9: 2-DG uptake in soleus muscle for the six treatment groups. All data is expressed as mean ± SEM. Asterisks above bars indicate significance between saline and glucose groups. The pound symbol (#) indicates significance between glucose and butyrate groups. The encircled letter (@) indicates significance between saline and butyrate groups. All significant groups are reported at p< 0.05 (n =19). Exact measurements and SEM values are reported in Table 3. Data is normalized to testis uptake.](image-url)
**Gastrocnemius muscle results:** As shown in Figure 10, glucose treatment significantly increased 2-DG uptake for all groups. There was no significant difference seen between saline-treated and butyrate-treated mice for the sedentary state. However, a 15-minute swim did produce a significant reduction in butyrate-treated animals.

![Figure 10: 2-DG uptake in gastrocnemius muscle for the six treatment groups. All data is expressed as mean ± SEM. Asterisks above bars indicate significance between saline and glucose groups. The pound symbol (#) indicates significance between glucose and butyrate groups. The encircled letter (@) indicates significance between saline and butyrate groups. All significant groups are reported at p< 0.05 (n = 19). Exact measurements and SEM values are reported in Table 3. Data is normalized to testis uptake.](image-url)
**Adipose tissue results:** As shown in Figure 11, glucose significantly increased 2-DG uptake in exercised mice.

![Figure 11: 2-DG uptake in adipose tissue for the six treatment groups. All data is expressed as mean ± SEM. Asterisk above bars indicates significance between saline and glucose groups. All significant groups are reported at p< 0.05 (n = 19). Exact measurements and SEM values are reported in Table 3. Data is normalized to testis uptake.](image)

**Muscle fiber comparison:** Since soleus and EDL muscles are predominantly composed of SO and FG fibers respectively, these two muscle groups provide information that allows fiber-specific comparisons. Because of insufficient
data, EDL comparisons could not be made for the sedentary animals. However, in exercised mice, comparisons were made between soleus and EDL for the different caloric input groups. As shown in Figure 12, the increase in 2-DG uptake produced by glucose treatment for EDL was significantly more dramatic than that produced in soleus. In contrast, the decrease in 2-DG uptake in soleus was significantly more dramatic than seen in EDL.

Figure 12: Comparison of soleus and EDL muscles under exercise conditions. Asterisks indicate significance between saline-treated soleus and EDL muscles. The pound symbol (#) indicate significance between glucose-treated soleus and EDL muscles. The encircled letter (@) indicate significance between butyrate-treated soleus and EDL muscles. Significance is reported at p <0.05 (n = 19). Exact measurements and SEM values are reported in Table 3. Data is normalized to testis uptake.
Discussion

An aim of these experiments was to evaluate the role of butyrate and exercise on 2-DG uptake in skeletal muscle and adipose tissue. In all statistically significant comparisons, glucose treatment caused an increase in 2-DG uptake for all skeletal muscles. This was as expected in that glucose caused the secretion of insulin which activated the translocation of GLUT4 to skeletal muscle membranes. Various other tissues responded differently to butyrate and exercise treatments as outlined below.

The gastrocnemius is composed predominantly of FG and FOG fibers, with 6-12% being SO fibers (Hargreaves, 1999). The gastrocnemius showed increases in 2-DG uptake under exercise conditions for saline and glucose diets (See Figure 10). Since saline and 2-DG did not elicit an insulin response, it is clear that contraction of the gastrocnemius resulted in higher 2-DG uptake when compared to controls (Figure 10). This would indicate GLUT4 transport. It is also evident that glucose and exercise resulted in additive 2-DG uptake when addition of saline-exercise and glucose-sedentary groups were compared to the glucose-exercise group. This additive effect was calculated to be 107%, which corresponds well with the data gathered by Gao and others (Gao, et al., 1994).

Under exercise conditions, there should be elevation in 2-DG uptake, since contraction causes an increase in GLUT4 content in an insulin-independent manner (Lund, et al., 1995). These experiments showed a decrease in 2-DG uptake in response to exercise in the soleus muscle. The most likely explanation is that under
the conditions of swimming, the soleus was underutilized relative to the "sedentary" state. In the experiments of Lund et al. (1995) the soleus was directly stimulated to contract ex vivo. In the experiments reported here, it is not known whether the soleus is actively contracting during a swimming exercise. One might expect that since its primary function is to counteract gravity, it may be relatively relaxed while swimming in conjunction with contraction by adjacent muscle groups such as the gastrocnemius. Another less likely possibility is that the soleus muscle could be outcompeted by other organs, such as the heart and brain, for the 2-deoxyglucose. However, the testis showed no change between exercise and sedentary conditions (see Appendix I) and would be equally outcompeted by more active tissues if that were the case. Finally, hormonal or internal transduction pathways in skeletal muscle could be responsible for these results, but it is uncertain what proteins may play a part in this role. From these hypotheses it is clear that more experiments could be conducted to resolve the questions concerning the soleus muscle.

Caloric source exhibited contrasting effects on various muscle types. The most distinctive comparisons would be between a muscle type that is predominantly FG and one that is SO, represented in these experiments by comparisons between EDL and soleus respectively. The data is significant for the exercised mice, which one could presume reflects the effects of caloric source against the background of contraction-stimulated uptake of 2-DG (See Figure 12). In these data, glucose stimulated 2-DG uptake as expected if insulin levels rose in response to higher than normal glucose levels in the bloodstream. This response is more dramatic in
EDL (FG) than in soleus (SO). Butyrate on the other hand causes a dramatic decrease in 2-DG uptake in soleus muscle (SO) and a less dramatic decrease in EDL (FG).

An explanation may be found in the conditions of the experiment. In the fasting condition prior to the exercise there may have been elevated levels of FFA in the bloodstream in response to glucagons and cortisol stimulation. Since the butyrate effect is to decrease soleus muscle 2-DG uptake possibly through metabolic inhibition of hexokinase, then having elevated FFA just immediately prior to treatment would have the greatest effect on soleus uptake as was observed in these experiments. Since EDL is primarily FG fibers, 2-DG uptake was dramatically increased by glucose treatment.

This still leaves unanswered why there is such a significant effect of butyrate on EDL. If the action of butyrate is through inhibition of hexokinase which blocks glycolysis through products of mitochondrial-mediated beta oxidation (acetylCoA and citrate), then the absence or paucity of mitochondria in the fibers in this muscle would argue against a significant effect by butyrate. It may be that butyrate’s effect is through some other undescribed mechanism in such muscle types, which may be a subject of future research.

Adipose tissue has different physiological functions from skeletal muscle. It is the responsibility of adipose tissue to supply substrates to other tissues when there is a demand. Exercise can be considered as one of these demands. Exercise
can cause lipolysis and glycogen breakdown in adipose tissue (Sherwood, 1997).

From Figure 11, the combination of saline and exercise showed a decrease in 2-DG uptake when compared to the saline sedentary group. This result could suggest competition for the uptake of 2-DG by other peripheral tissues, such as the gastrocnemius and EDL. However for the same reasons outlined above for soleus decrease under exercise conditions, the testis controls argue against competition for 2-DG. Under exercise conditions, the blood glucose levels would be driven even lower than sedentary after a 12-hour fast, and as a consequence of glucagon action, adipose tissue may respond in a way that is the opposite of that experienced under insulin stimulation as seen for several other metabolic cycles.
Conclusion

Evidence has shown that high fat diets for extended amounts of time will cause insulin resistance in skeletal muscle and lipogenesis in adipose tissue resulting in obesity-related Type 2 Diabetes. The purpose of this study was to evaluate the role of butyrate and exercise on glucose uptake in skeletal muscle and adipose tissue under a single bout of swimming exercise.

The study has shown:
1) *In vivo*, glucose and exercise provided a 107% additive effect on 2-DG uptake in mice.
2) The soleus muscle showed a decrease in 2-DG uptake in a 15-minute swim compared to sedentary controls under three different caloric sources.
3) A large portion of skeletal muscle showed a decrease in 2-DG uptake under butyrate conditions for both sedentary and exercise regiments.
4) Muscle fiber types play an important role in 2-DG uptake. Fast twitch fibers (e.g. EDL) had higher 2-DG uptakes than slow twitch fibers (e.g. soleus) under exercise conditions.
5) Contraction caused glucose uptake in fast twitch muscles under non-insulin stimulated conditions.

The hypothesis for my experiments was that the combination of butyrate and exercise will impair glucose uptake in skeletal muscle and enhance glucose uptake in adipose tissue. From these results, it is conclusive that butyrate and exercise decrease 2-DG uptake among control groups in skeletal muscles (54% soleus, 20% EDL, and 42% gastrocnemius). In adipose tissue, however, significance between sedentary, saline and exercise, saline mice could only be established with exercise treatments having lower 2-DG uptake than sedentary controls. These results help to validate intraperitoneal 2-DG injections as a murine model of study for the effects of caloric source and exercise on glucose metabolism.
# Appendix One

## Saline Sedentary Group

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### Saline Exercise Group

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### Tissue weight

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