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

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Master of Science in Biology

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by

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Director of Thesis

Master's Committee

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Director of Thesis: _____

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}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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I would like to express thanks to Dr. Lorraine Nolte for her technical advice on skeletal muscle *in vitro* incubations and taking time out of her busy schedule to answer my questions via e-mail.

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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).

Isoform	Tissue Distribution	Function
GLUT1	Ubiquitous. High expression in brain endothelial cells and human erythrocytes	Basal glucose transport Located in plasma membrane Low K_m (2-3 mM)
GLUT2	Liver, kidney and small epithelial cells, β-cells of pancreas	High K_m (15-20 mM) Glucose transport in and out of hepatocytes and β-cells
GLUT3	Neurons, placenta	Low K_m (2-3 mM) Transport into CNS
GLUT4	Skeletal muscle, brown and white adipose tissue, heart	Mediates insulin and contraction regulated glucose transport K_m (5-6 mM)
GLUT5	Small intestine, kidney, skeletal muscle, human adipocytes	Fructose transporter
GLUT7	Liver	Part of the glucose-6-phosphatase complex in endoplasmic reticulum

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).

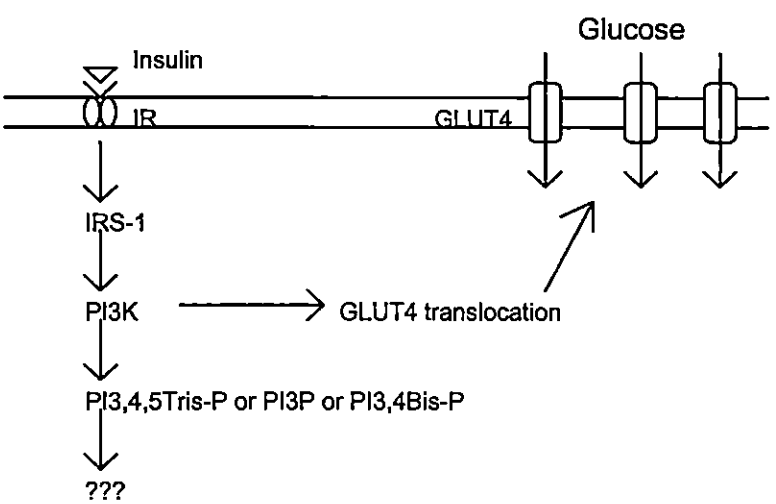

Figure 1: Insulin signaling cascade in skeletal muscle (taken from Hansen, et al., 1996)

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.)

Characteristic	*Type of Fiber*		
	Slow-oxidative (SO)	Fast-oxidative glycolytic (FOG)	Fast-glycolytic (FG)
Myosin-ATPase activity	Low	High	High
Speed of Contraction	Slow	Fast	Fast
Presence of Mitochondria	Many	Many	Few
Capillaries	Many	Many	Few
Myoglobin Content	High	High	Low
Color of Fiber	Red	Red	White
Resistance to Fatigue	High	Intermediate	Low
Glycogen Storage	Low	Intermediate	High
Oxidative-Phosphorylation	High	High	Low

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 ($VO_{2\ max}$). The $VO_{2\ max}$ quantitates the intensity of exercise which plays a significant role in muscle performance (Hawley, et al., 1997). A high $VO_{2\ max}$ (80-90%) indicates a high-intensity workout and will result in early FG fiber fatigue. A low $VO_{2\ max}$ (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.

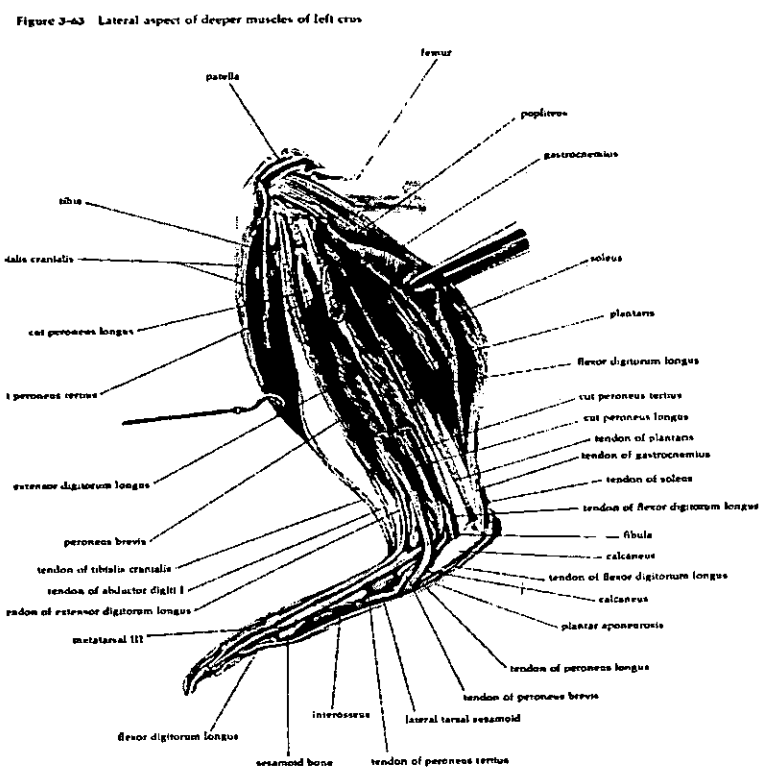

Figure 2: Anatomical diagram of hindlimb muscles in the guinea pig (taken from Cooper, 1975)

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-CoAs enter the citric acid cycle (TCA) and generate NADH/$FADH_2$, 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) = Abs. of sample/Abs. of standard x 100

Intraperitoneal Injections of (^{14}C) 2-Deoxyglucose

Mice were injected intraperitoneally (1 cc syringe with a 27-gauge needle) with 10 μCi of (^{14}C) 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 $FADH_2$, 1 NADH, and 2 acetyl-CoA (2.5 ATP = 1 NADH and 1.5 ATP = 1 $FADH_2$). 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.

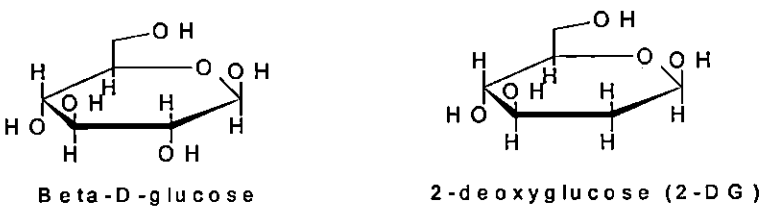

Figure 3: Structural differences between 2-DG and β-D-glucose.

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 scapel 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 scapel. 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 scapel 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.

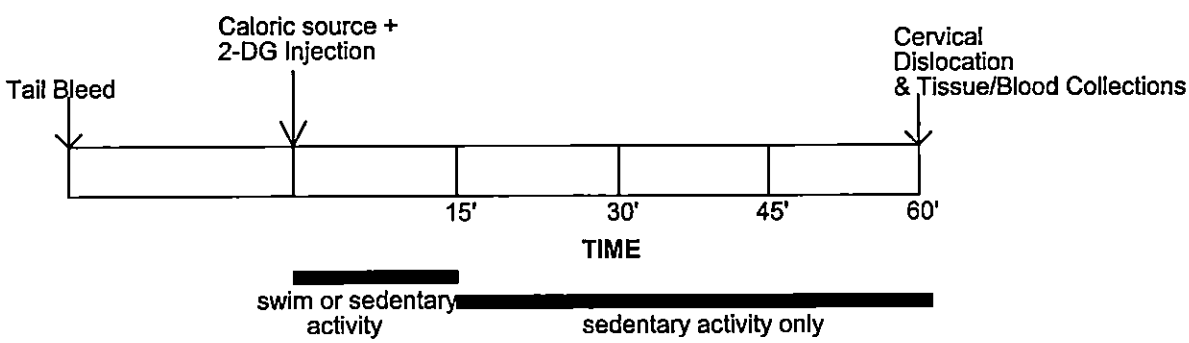

Figure 4: Diagram of experimental design for intraperitoneal injections

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.

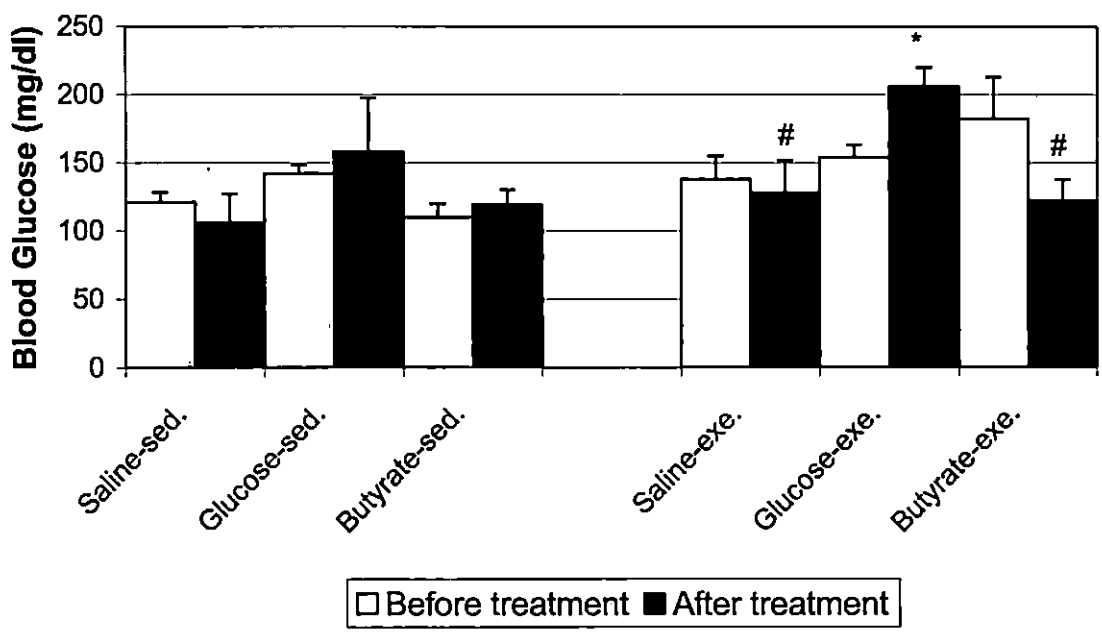

Figure 5: Blood glucose determinations before and after treatments in saline, glucose, and butyrate-treated mice. Sedentary and exercise groups are indicated as "sed." and "exe." Asterisks indicate significance between before and after 2-DG injections in glucose exercise mice. A comparison was made between 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.

		Saline	Glucose	Butyrate	Signific. of Diet* ($p < 0.05$)	Signific. of Exercise* ($p<0.05$)
Soleus	Nonexercise	7.44 ± 0.41	11.38 ± 0.47	3.99 ± 0.55	yes	
Soleus	Exercise	3.39 ± 0.39	4.64 ± 0.42	0.632 ± 0.15	yes	yes
Liver	Nonexercise	0.28 ± 0.27	0.807 ± 0.48	0.431 ± 0.33	no	
Liver	Exercise	0.55 ± 0.25	1.07 ± 0.94	3.71 ± 1.80	yes	yes, B
Kidney	Nonexercise	0.781± 0.71	0.807 ± 0.48	0.904 ± 0.80	no	
Kidney	Exercise	0.486 ± 0.2	3.15 ± 1.61	2.74 ± 0.60	no	yes, S & G
Gastroc	Nonexercise	0.717 ± 0.14	1.29 ± 0.04	0.599 ± 0.12	yes, G vs S,B	
Gastroc	Exercise	0.974 ± 0.14	2.11 ± 0.09	0.406 ± 0.10	yes	yes, G
EDL•	Nonexercise	1.08	13.3	1.3		
EDL	Exercise	4.12 ± 0.22	7.49 ± 0.87	2.73 ± 0.42	yes	no
Anterior Tib.	Nonexercise	0.87 ± 0.04	1.80 ± 1.27	2.77 ± 0.49	no	
Anterior Tib.	Exercise	2.98 ± 0.76	3.99 ± 0.64	1.33 ± 0.56	no	yes
Adipose	Nonexercise	0.549 ± 0.1	0.563 ± 0.05	0.751 ± 0.10	no	
Adipose	Exercise	0.201 ± 0.1	0.744 ± 0.09	0.979 ± 1.45	yes S& G	yes, S
Heart	Nonexercise	1.52 ± 0.12	4.0 ± 2.17	3.66 ± 0.425	no	
Heart	Exercise	3.56 ± 1.67	3.66 ± 1.38	1.31 ± 1.10	no	no
Large Int.	Nonexercise	0.816 ± 0.6	1.66 ± 0.47	0.446 ± 0.41	no	
Large Int.	Exercise	2.53 ± 1.0	1.02 ± 0.51	0.615 ± 0.32	no	no

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.

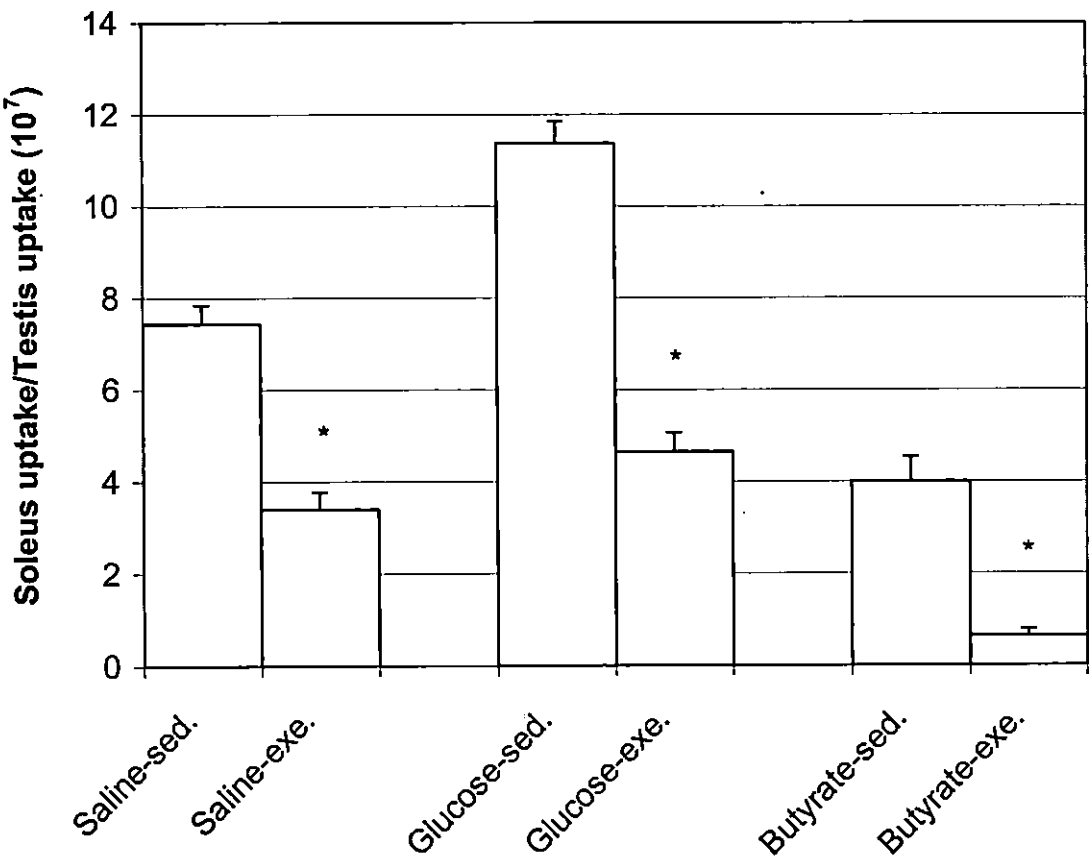

Figure 6: 2-DG uptake in soleus muscle for the six treatment groups. All data is expressed as mean $\pm$ SEM. Asterisks above the bars are significant in comparison of sedentary versus exercise groups. Significance is at $p < 0.05$ ($n = 10$). Exact values and SEM measurements are reported in Table 3. 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.

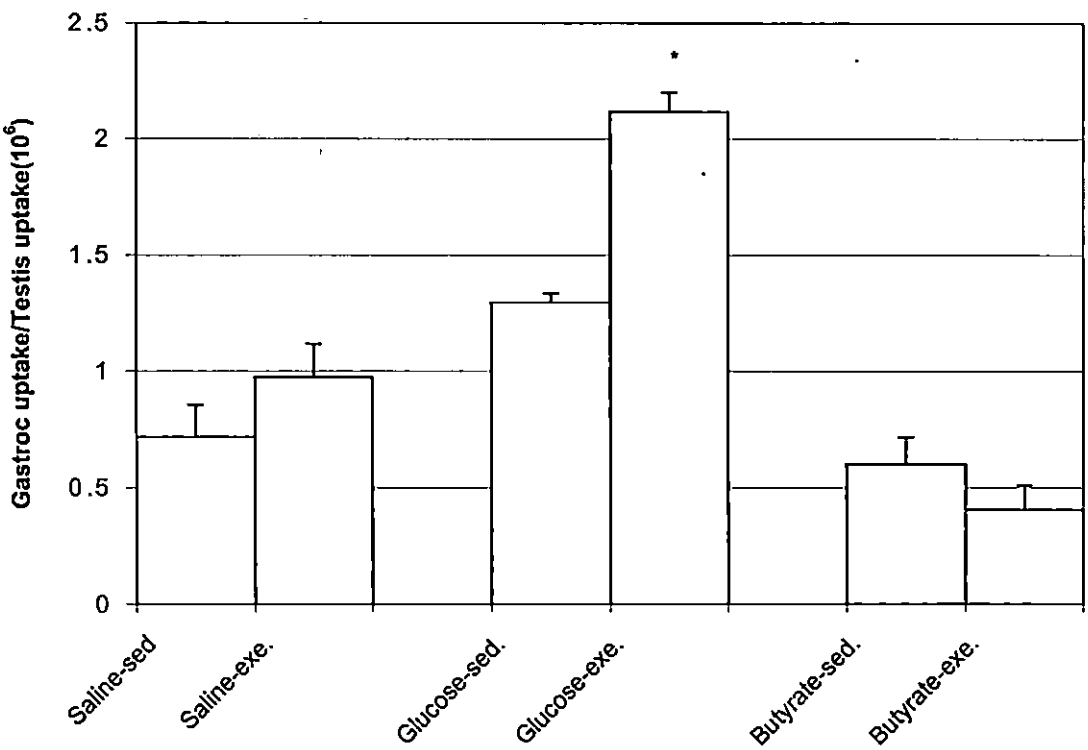

Figure 7: 2-DG uptake in gastrocnemius muscle for the six treatment groups. All data is expressed as mean $\pm$ SEM. Asterisks above the bars are significant in comparison of sedentary versus exercise groups. Significance is at $p < 0.05$ ($n = 10$). Exact measurements and SEM values are reported in Table 3. Data is normalized to testis uptake.

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.

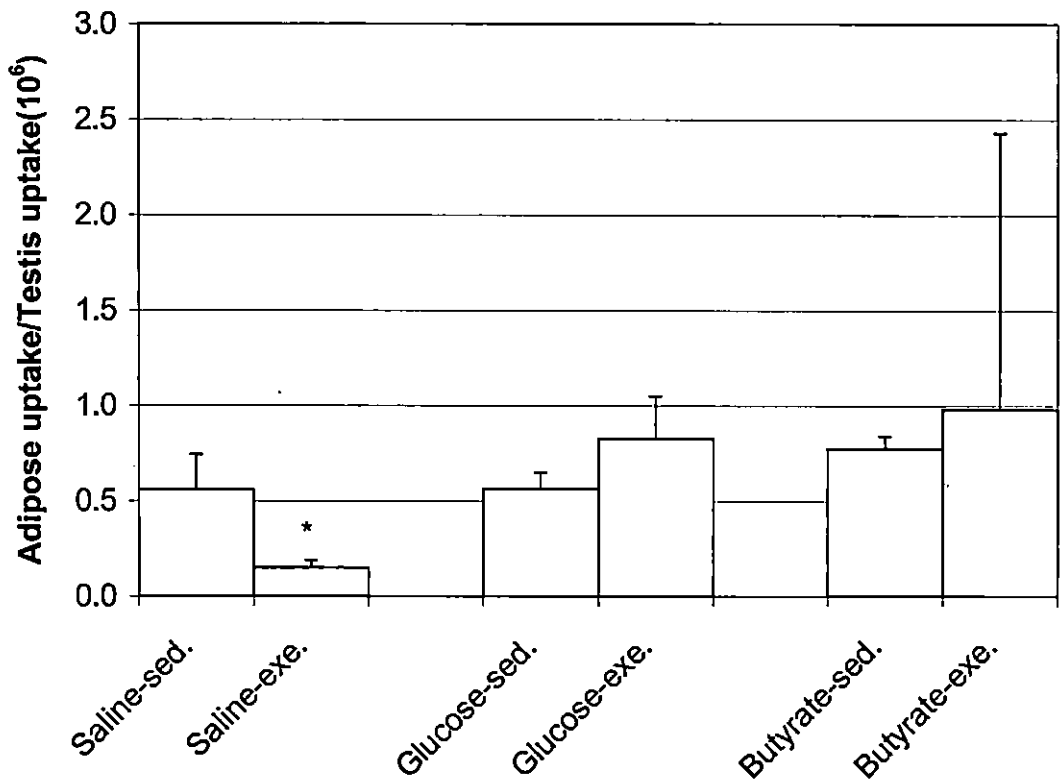

Figure 8: 2-DG uptake in adipose tissue for the six treatment groups. All data is expressed as mean $\pm$ SEM. Asterisks above the bars are significant in comparison of sedentary versus exercise groups. Significance is at $p < 0.05$ ($n = 10$). Exact measurements and SEM values are reported in Table 3. Data is normalized to testis uptake.

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.

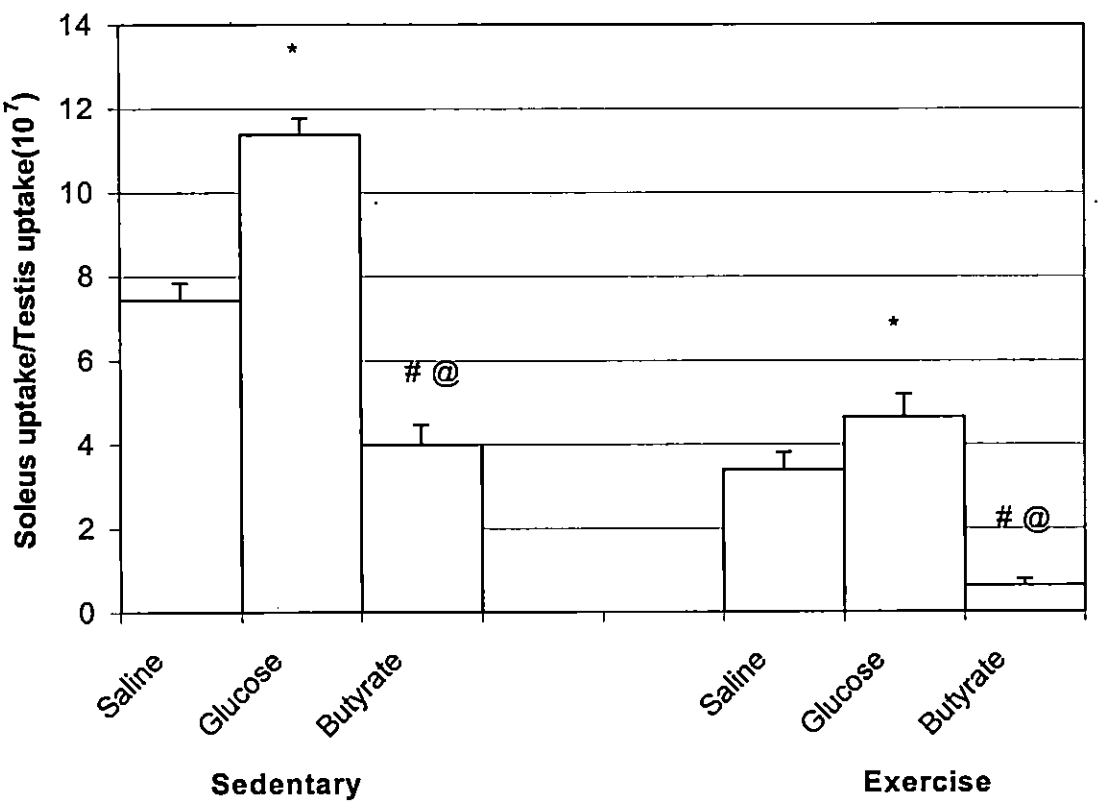

Figure 9: 2-DG uptake in soleus muscle for the six treatment groups. All data is expressed as mean ± SEM. Asterisks above bars indicate significance between saline and glucose groups. The pound symbol (#) indicates significance between glucose and butyrate groups. The encircled letter (@) indicates significance 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.

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.

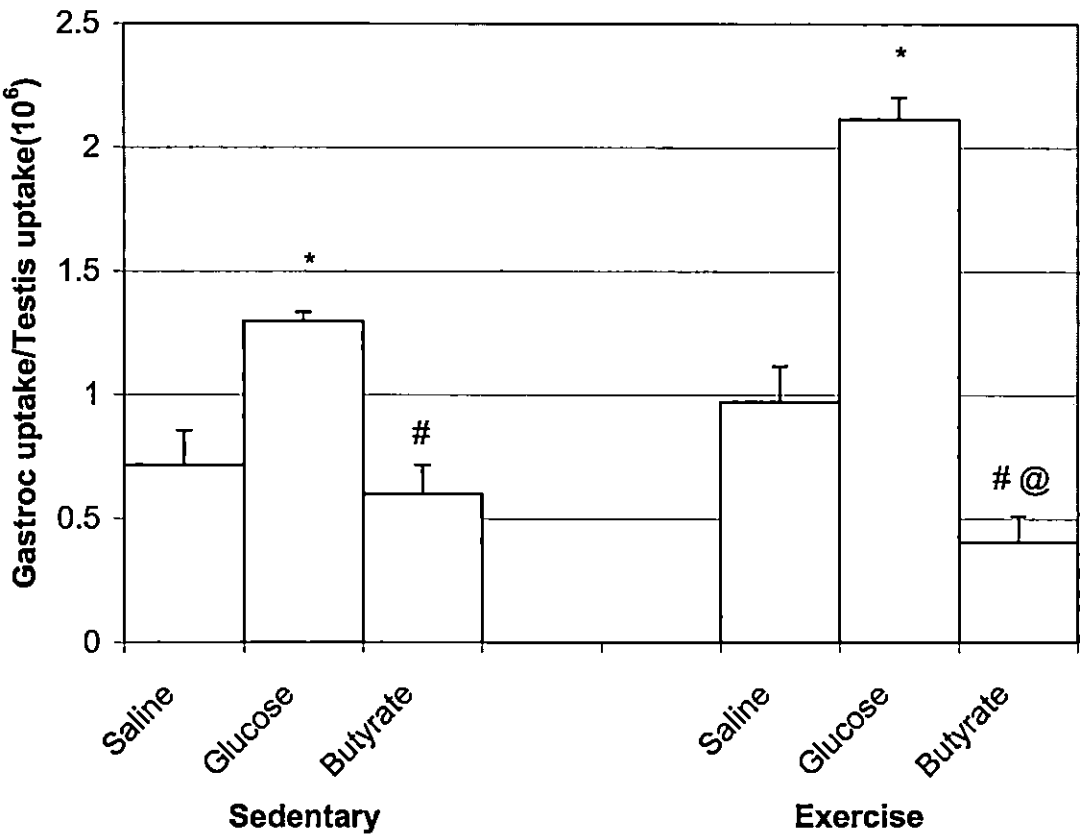

Figure 10: 2-DG uptake in gastrocnemius muscle for the six treatment groups. All data is expressed as mean ± SEM. Asterisks above bars indicate significance between saline and glucose groups. The pound symbol (#) indicates significance between glucose and butyrate groups. The encircled letter (@) indicates 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.

Adipose tissue results: As shown in Figure 11, glucose significantly increased 2-DG uptake in exercised mice.

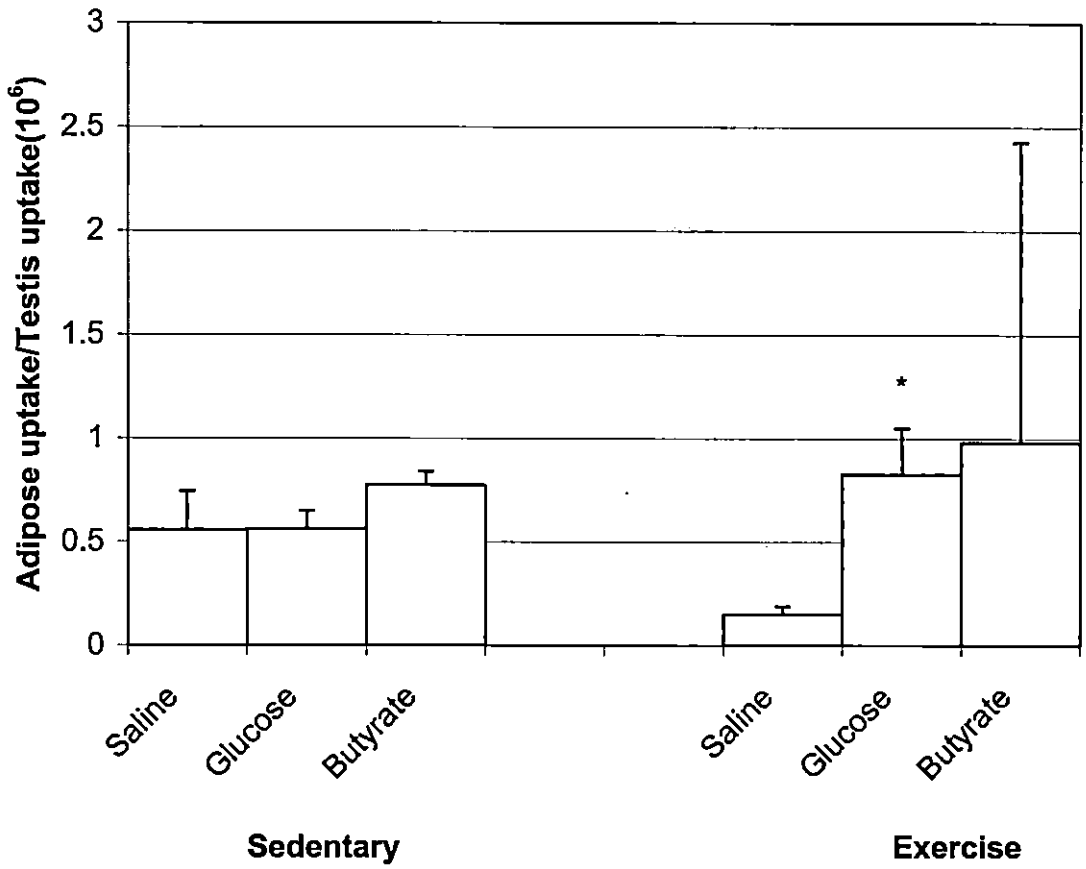

Figure 11: 2-DG uptake in adipose tissue for the six treatment groups. All data is expressed as mean $\pm$ SEM. Asterisk above bars indicates significance between saline and glucose groups. All significant groups are reported at $p < 0.05$ ($n = 19$). Exact measurements and SEM values are reported in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

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.

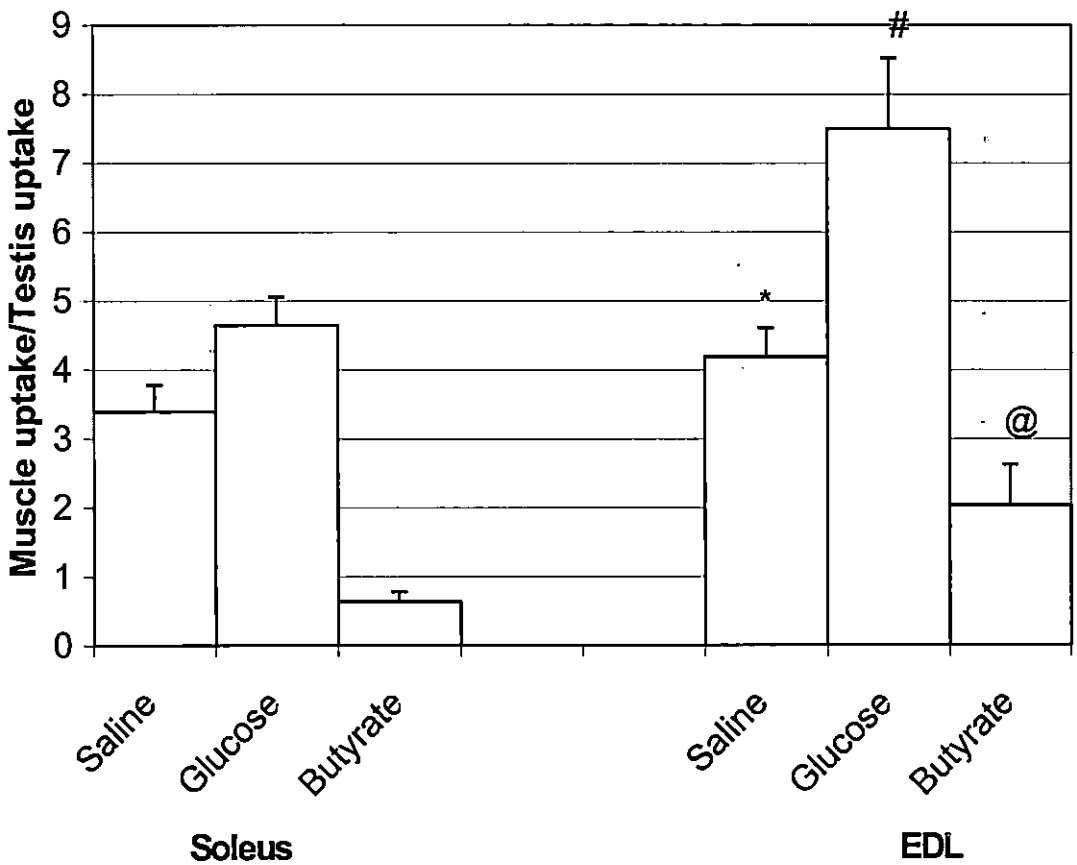

Figure 12: Comparison of soleus and EDL muscles under exercise conditions. Asterisks indicate significance between saline-treated soleus and EDL muscles. The pound symbol (#) indicate significance between glucose-treated soleus and EDL muscles. The encircled letter (@) indicate significance between butyrate-treated 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

	Mouse 1		Mouse 2		Mouse 3
soleus	2849.01		2697.33		2425.54
soleus	3291.8				3108
brain	10215				2845.92
liver	3789.06		3543.51		8892.17
large int.	13501.33		6607.1		12546.25
testis	11222.22		9504.54		11685.55
heart	23344.44		16980		23608.89
kidney	15493.85		13733.33		18105
kidney			16029.23		
adipose	8682.5		7278.57		10705.26
ant. Tibialis	4025.2		3363.33		10564.21
gastroc	3225.08		3743.7		11635.55
EDL	949				
Liver	5076.5		7500.74		6102.42
Tissue weight					
soleus	0.0033		0.007		0.0033
soleus	0.0044				0.0051
brain	0.004				0.0389
liver	0.1072		0.1399		0.3393
large int.	0.1186		0.073		0.2693
testis	0.0961		0.1004		0.1297
heart	0.1163		0.1168		0.1995
kidney	0.159		0.2188		0.2302
kidney			0.2241		
adipose	0.1118		0.1673		0.2274
ant. Tibialis	0.0433		0.039		0.107
gastroc	0.0538		0.0849		0.1851
EDL	0.0075				
Liver	0.1072		0.18		0.2631

Saline Exercise Group

	Mouse 1		Mouse2		Mouse 3		Mouse 4
soleus	672		1783.72		1384.28		296.8
soleus	549		688.8		1277.71		236.1
liver	7738.46		12596.25		11005.26		2437.11
liver			4206.67		7079.31		767.6
large int.	14257.33		84733.33		109653.3		7550.37
testis	8921.74		10924.21		11161.11		4805.24
testis	8289.6		9514.54		10238		4169.17
heart	10591.58		43732		87380		4974.15
kidney	10890.53		71153.33		62830		4814.29
kidney	14382.86		71020		61790		4516.89
adipose	4747.44		1356.89		1488.44		1258.74
ant. Tibialis	5912.35		21988		10237		6783.33
ant. Tibialis			15873.85		9722.86		3236.45
gastroc			8478.33		8220		2860.57
gastroc	8788.7		7607.41		7793.08		2712.16
EDL			1187.34		2020		1421.28
EDL			567		1266.92		1076.56
stomach	7984.61						
Tissue weight							
soleus	0.0032		0.0067		0.0075		0.0062
soleus	0.0024		0.0014		0.0042		0.005
liver	0.3361		0.2045		0.1805		0.2823
liver			0.047		0.0915		0.0945
large int.	0.3427		0.1676		0.3605		0.2051
testis	0.1464		0.1084		0.1283		0.1225
testis	0.0966		0.0965		0.125		0.1074
heart	0.1196		0.115		0.1223		0.1554
kidney	0.2094		0.2425		0.2453		0.2531
kidney	0.2291		0.2165		0.2314		0.2503
adipose	0.372		0.175				0.1012
ant. Tibialis	0.3986		0.0375		0.0413		0.0391
ant. Tibialis			0.0296		0.0373		0.0382
gastroc	0.2196		0.1155		0.1172		0.1038
gastroc			0.11		0.1112		0.0948
EDL			0.0052		0.0065		0.0063
EDL			0.0032		0.0053		0.0056
stomach	0.204						

Glucose Sedentary Group

	Mouse 1		Mouse 2		Mouse 3
soleus	1800.89		2570.51		2518
soleus	1541.38		2471.11		2458.29
liver	17170		5720		5370.53
liver	13355		1509.47		2515.75
large int.	11252.22		7785.38		21322
testis	7639.26		5400.53		3319.02
testis	6509.03		5480.54		4882.86
heart	7285		18565.46		46112
kidney			5068.5		3781.51
kidney	89453.33		4097.55		4229.17
adipose	10078		4289.79		3268.71
ant. Tibialis	2899.14		7082.76		
gastroc	9817.14		7170		4738.6
EDL			487		
stomach					16313.85
Tissue Weight					
soleus	0.0022		0.0039		0.0051
soleus	0.0008		0.0016		0.0047
liver	0.1676		0.1634		0.2529
liver	0.1277		0.0752		0.0923
large int.	0.3277		0.2244		0.1277
testis	0.0972		0.0887		0.0791
testis	0.0881		0.0964		0.1232
heart	0.1105		0.1013		0.1346
kidney	0.1936		0.1773		0.1838
kidney			0.1663		0.1931
adipose	0.2662		0.1287		0.1178
ant. Tibialis	0.0324		0.0273		
gastroc	0.1014		0.0904		0.0829
EDL			0.0006		
stomach					0.2501

Glucose Exercise Group

	Mouse 1		Mouse2		Mouse3
soleus	1138.75		1136.05		1087.39
soleus	785.9		828.1		
liver	10882.11		19980		8739.13
liver	6834.67		7377.86		8464.17
large int.	16147.69		3878.46		12295.29
testis	7000		7759.23		9267.27
testis	6857.33				8839.13
heart	16041.54		23235.55		47512
kidney	64420		26502.5		21990
kidney	63545		26037.5		15532.31
adipose	3648.73		4439.57		2766.03
ant. Tibialis			13666.67		12758.75
ant. Tibialis			6081.21		8156.8
gastroc	12956.25		16461.54		15680
gastroc	12742.5		16455.38		13868
EDL	3088.62		2833.24		3727.41
EDL	1881.87		1638.7		3266.77
Tissue weight					
soleus	0.004		0.0053		0.0012
soleus	0.0028		0.0034		
liver	0.2276		0.2928		0.1974
liver	0.0859		0.1057		0.1405
large int.	0.1931		0.3372		0.1924
testis	0.16		0.1075		0.145
testis	0.1187				0.0985
heart	0.1746		0.1302		0.1159
kidney	0.2459		0.2265		0.1788
kidney	0.2297		0.2246		0.1677
adipose	0.06		0.1133		0.0444
ant. Tibialis			0.0543		0.035
ant. Tibialis			0.0318		0.031
gastroc	0.1203		0.1295		0.1142
gastroc	0.1123		0.1234		0.0961
EDL	0.0092		0.0071		0.006
EDL	0.006		0.0044		0.005

Butyrate Sedentary Group

	Mouse 1 (B1)		Mouse 2 (B2)		Mouse 3 (B3)
soleus	1826.73		1807.93		1196.55
soleus			1209.76		685.1
liver	12250.59		8190.4		8994.78
liver	2184.57		3901.54		5558.38
large int.	8255.2		11397.78		13782.67
testis	9240		8148		11880
testis	7512.59		6627.1		10598.95
heart	44724		29808.57		32888.57
kidney	13661.33		11046.32		14317.14
kidney	11421.11		10227		13702.67
adipose	2119.79		4854.76		2252.81
ant. Tibialis	6871.33		9754.29		9700
gastroc	5689.44		2454.88		4433.04
EDL	513				
stomach			9422.73		21484
upper leg	7128.28				
Tissue Weight					
soleus	0.0024		0.0076		0.0045
soleus			0.0039		0.0025
liver	0.2752		0.2699		0.2348
liver	0.0716		0.2217		0.1951
large int.	0.3207		0.3864		0.3892
testis	0.1456		0.1433		0.1391
testis	0.1407		0.1306		0.1391
heart	0.1625		0.1389		0.1339
kidney	0.1888		0.229		0.2087
kidney	0.182		0.2104		0.2027
adipose	0.0388		0.1017		0.0478
ant. Tibialis	0.0541		0.0466		0.043
gastroc	0.1079		0.096		0.1005
EDL	0.0062				
stomach			0.148		0.249
uppper leg	0.211				

Butyrate Exercise Group

	Mouse 1		Mouse 2		Mouse 3
soleus	945		186.7		507.2
soleus	347.4		166.2		470.9
liver	8092		896		25670
liver	3108.62		617.5		10186
large int.	4133.06		39540		14694.29
testis	4030.4		343		12856.25
testis	3699.64		314.4		10813.68
heart	6325.62		530		35556.66
kidney	7365.71		1047.33		61830
kidney	6903.45		1036.68		54700
adipose	439.7		217.9		11001.05
ant. Tibialis	5240.51		456.2		11742.22
ant. Tibialis	3581.07		447		5981.76
gastroc	8652.5		1193.26		4376.96
gastroc	8410.83		928.1		2977.06
EDL	2499.51		168		1686.05
EDL	1112.11		163.6		1223.9
Tissue wt.					
soleus	0.0062		0.0062		0.0063
soleus	0.0057		0.006		0.0058
liver	0.3142		0.1962		0.2281
liver	0.1173		0.0915		0.0085
large int.	0.3255		0.3685		0.342
testis	0.1573		0.1584		0.14
testis	0.1342		0.1198		0.1307
heart	0.1487		0.132		0.1229
kidney	0.2126		0.2267		0.2621
kidney	0.197		0.2202		0.2402
adipose	0.0409		0.0821		0.1136
ant. Tibialis	0.0553		0.0327		0.0422
ant. Tibialis	0.0442		0.035		0.0269
gastroc	0.1318		0.1257		0.1253
gastroc	0.1082		0.1058		0.0725
EDL	0.0062		0.0056		0.0065
EDL	0.0056		0.0056		0.0051

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