Functional Overload-Induced Muscle Hypertrophy and Glucose Uptake Occurs Independent of Glucose Transporter 4

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INTRODUCTION

Functional overload induces a number of adaptations in skeletal muscle that are similar to resistance exercise training, including muscle hypertrophy and glucose uptake. While numerous studies have investigated the molecular/ cellular mechanisms underlying overload-induced muscle growth, little is known regarding the mechanism(s) underlying overload-induced glucose uptake.

Aim1: Determine whether overload-induced skeletal muscle glucose uptake is muscle fiber type dependent.

METHODS/RESULTS:

– Female CD-1 mice underwent unilateral synergist ablation surgery to induce functional overload of the plantaris or soleus muscle. After 5 days muscles were weighed, and then incubated in [3H]-2-deoxyglucose to assess glucose uptake.

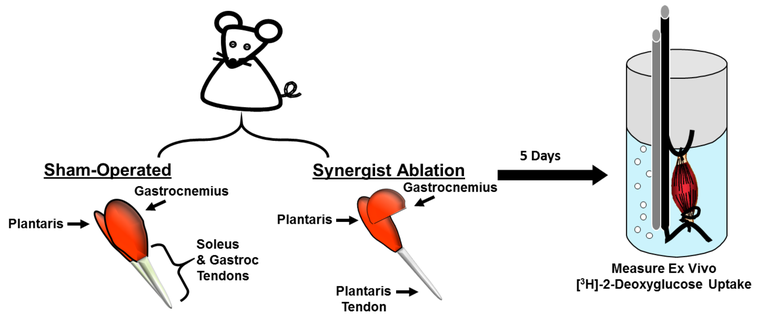


Fig 1. Model of Unilateral Synergist Ablation Surgery and Ex Vivo Skeletal Muscle Glucose Uptake.

A)

Muscle	Weight (mg)
Plantaris	
-sham	11.1 ± 0.53
-OVL	17.5 ± 0.58[a]
Soleus	
-sham	7.8 ± 0.45
-OVL	10.5 ± 0.82[a]

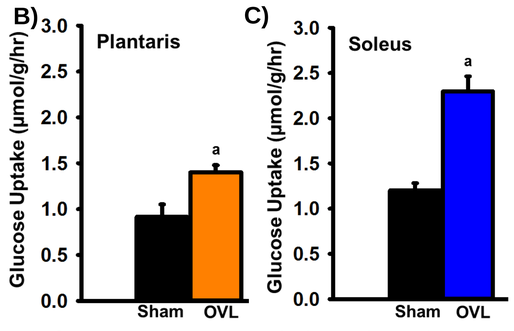


Fig 2. Overload-induced glucose uptake occurs independent of muscle fiber type. (A) Muscle weights in the sham and overloaded (OVL) conditions. Overload-induced [3H]-2-deoxy-D-glucose uptake in (B) plantaris and (C) soleus muscles (P<0.05 = 'a' vs sham. N= 4-6 muscle/group.)

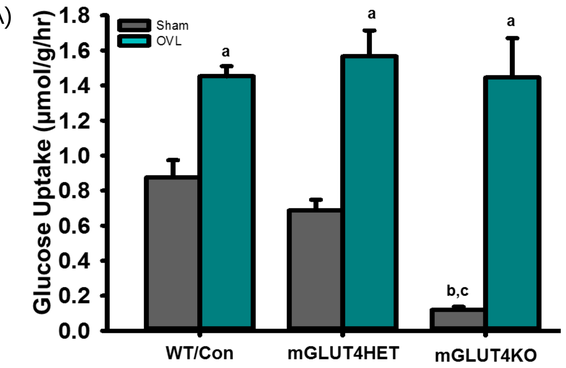
Aim 2: Determine whether GLUT4 regulates overload-induced skeletal muscle glucose uptake.

Glucose transporter 4 (GLUT4) is the predominant glucose transporter in muscle and mediates muscle glucose uptake in response to insulin and muscle contraction (Zisman et al., Nat Med. 6:924-8, 2000). However, its role in overload-induced glucose uptake is currently unknown.

METHODS/RESULTS:

– GLUT4 LoxP mice (donated by Dr. B. Kahn) were bred to muscle creatine kinase Cre recombinase mice to produce wild-type (WT)/control (CON; Cre+, LOXP+), muscle specific GLUT4 heterozygous (mGLUT4HET), and muscle specific GLUT4 knockout mice (mGLUT4KO). Plantaris overload was induced by unilateral synergist ablation, and [3H]-2-deoxyglucose uptake was assessed 5 days later.

A)



B)

Genotype	Plantaris muscle weight (mg)
WT/CON	
-sham	12.5 ± 0.3
-OVL	18.8 ± 0.4[a]
mGLUT4HET	
-sham	12.4 ± 0.3
-OVL	18.6 ± 0.7[a]
mGLUT4KO	
-sham	12.5 ± 0.4
-OVL	17.5 ± 0.8[a]

Fig 3. GLUT4 does not mediate overload-induced muscle glucose uptake. Sham muscle glucose uptake was impaired in the mGLUT4KO and mGLUT4HET compared to the WT/CON mice. (A) Muscle glucose uptake. (B) Plantaris muscle weight. (OVL = Overload) (a = P<0.05 vs Sham, b = P<0.05 vs WT/Con, c = P<0.05 vs mGLUT4HET).

Aim 3: Determine which transporter mediates overload-induced glucose uptake.

Skeletal muscle expresses both facilitative glucose transporters (GLUTs) and sodium-dependent glucose co-transporters (SGLTs).

METHODS/RESULTS:

- Transporter Inhibition: [3H]-2-deoxyglucose uptake assessed in the presence of the SGLT inhibitor, phloridzin or the GLUT inhibitor, cytochalasin B in 5 day overload-stimulated plantaris muscles from CD-1 female mice.

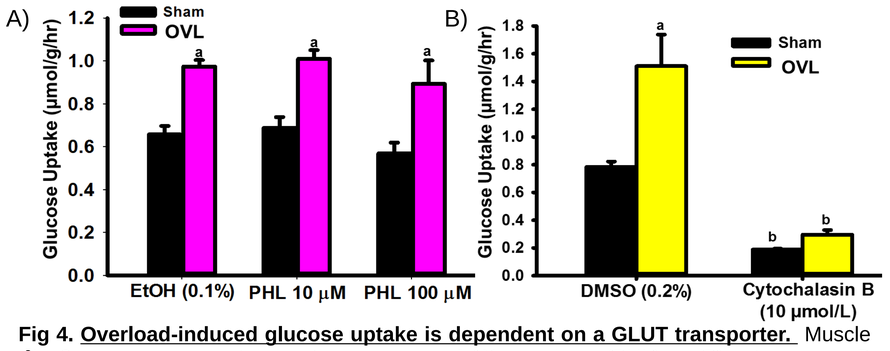
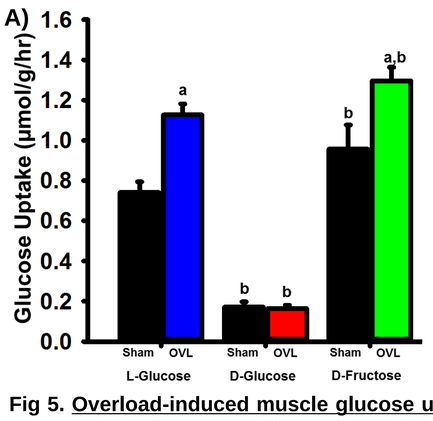


Fig 4. Overload-induced glucose uptake is dependent on a GLUT transporter. Muscle [3H]-2-deoxyglucose uptake in the presence of (A) phloridzin (PHL) or (B) cytochalasin B (P<0.05 = 'a' vs sham, 'b' vs vehicle (either DMSO or EtOH). N=4-6 muscles/ group.

To determine which GLUT isoform(s) is/are contributing to functional overload-induced muscle glucose uptake hexose competition assays were performed.

METHODS/RESULTS:

•Hexose Competition: CD-1 female mice underwent unilateral synergist ablation surgery to induce plantaris muscle hypertrophy. After 5 days, ex vivo muscle [3H]-2-deoxy-D-glucose uptake was assessed in the presence of 35 mM L-glucose, D-glucose, or D-fructose.



B)

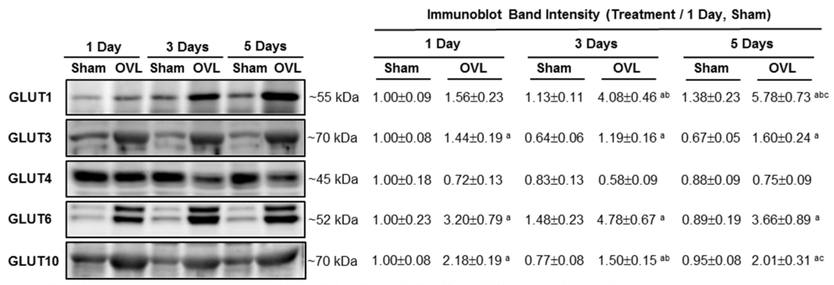
GLUT ISOFORM	Transports 2-deoxyglucose	Inhibited by Cyto B	Higher affinity for D-fructose vs 2-deoxyglucose
GLUT1	Yes	Yes	No
GLUT3	Yes	Yes	No
GLUT5	Yes	Yes	Yes
GLUT6	Yes	Yes	No
GLUT7	No	-	-
GLUT8	Yes	Yes	Yes
GLUT9	Yes	No	No
GLUT10	Yes	Yes	No
GLUT12	Yes	Yes	Yes
GLUT13	No	-	-

Fig 5. Overload-induced muscle glucose uptake is dependent on a GLUT(s) which transports D-glucose and has a low affinity for D-fructose. (A) Hexose competition. (B) GLUT isoforms that are in the mouse skeletal muscle and their substrate affinity characteristics. (P<0.05 = 'a' vs sham, 'b' vs L-glucose) N=4-6 muscles/group.

METHODS/RESULTS:

GLUT transporter protein expression was examined in the following conditions:

•Female wild-type mice underwent unilateral synergist ablation surgery to induce plantaris muscle hypertrophy. After 1, 3, or 5 days, muscles were processed to assess GLUT isoforms protein expression by immunoblot (IB) analysis.



	1 Day Sham	1 Day OVL	3 Days Sham	3 Days OVL	5 Days Sham	5 Days OVL
GLUT1	~55 kDa	1.00±0.09	1.56±0.23	1.13±0.11	4.08±0.46 ab	1.38±0.23	5.78±0.73 abc
GLUT3	~70 kDa	1.00±0.08	1.44±0.19 a	0.64±0.06	1.19±0.16 a	0.67±0.05	1.60±0.24 a
GLUT4	~45 kDa	1.00±0.18	0.72±0.13	0.83±0.13	0.58±0.09	0.88±0.09	0.75±0.09
GLUT6	~52 kDa	1.00±0.23	3.20±0.79 a	1.48±0.23	4.78±0.67 a	0.89±0.19	3.66±0.89 a
GLUT10	~70 kDa	1.00±0.08	2.18±0.19 a	0.77±0.08	1.50±0.15 ab	0.95±0.08	2.01±0.31 ac

Fig 6. Overload increased GLUT1, GLUT3, GLUT6 and GLUT10 protein expression in wild type mice. Representative blots and quantification provided above. Statistical significance was defined as P<0.05 and denoted by 'a' vs sham, 'b' vs 1 Day, 'c' vs 3 Days. N=6-7 muscles/group.

METHODS/RESULTS:

•Female wild-type/control (WT/CON), muscle-specific GLUT4 heterozygous (mGLUT4 HET), and muscle-specific GLUT4 knockout (mGLUT4 KO) mice underwent unilateral synergist ablation surgery to induce plantaris muscle hypertrophy. After 5 days, muscles were processed to assess GLUT isoform protein levels by immunoblot (IB) analysis.

	WT/CON Sham	WT/CON OVL	mGLUT4 HET Sham	mGLUT4 HET OVL	mGLUT4 KO Sham	mGLUT4 KO OVL
GLUT1	~55 kD	1.00±0.26	2.45±0.33 a	1.19±0.21	3.13±0.45 a	0.92±0.21	3.69±0.82 a
GLUT3	~70 kD	1.00±0.08	2.26±0.17 a	1.00±0.18	1.77±0.30 a	0.96±0.24	2.21±0.30 a
GLUT4	~45 kD	1.00±0.07	0.83±0.13	0.65±0.07 bd	0.66±0.13 d	0.06±0.01 bcde	0.05±0.02 bcde
GLUT6	~52 kD	1.00±0.31	2.60±0.52 a	0.66±0.05	1.95±0.08 a	0.48±0.10 d	1.52±0.26 abd
GLUT10	~70 kD	1.00±0.08	2.53±0.20 a	0.83±0.07	1.90±0.21 ab	0.90±0.13	2.75±0.35 ac

Fig 7. GLUT1, GLUT3, GLUT6, and GLUT10 protein expression increases following 5 days of functional overload in mice with muscle-specific loss of GLUT4. Statistical significance was defined as P<0.05 and denoted by 'a' vs sham, 'b' vs WT/CON, 'c' vs mGLUT4 HET, 'd' genotype main effect vs WT/CON, 'e' genotype main effect vs mGLUT4 HET. N=5-7 muscles/group.

Summary

Overload increases glucose uptake in both mouse soleus and plantaris muscle (i.e. independent of muscle fiber type).

Glucose transporter 4 (GLUT4) is not required for overload-induced muscle glucose uptake.

Overload-induced muscle glucose uptake is mediated by a facilitated glucose transporter isoform (GLUT) that has a higher affinity for D-glucose and a lower affinity for L-glucose and D-fructose compared to 2-deoxy-D-glucose.

GLUT1, GLUT3, GLUT6, and GLUT10 protein levels increase following overload in WT/CON and mGLUT4KO mice.

Conclusion

GLUT1, GLUT3, GLUT6 and/or GLUT10, are responsible for skeletal muscle glucose uptake in response to chronic muscle loading.

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