



University of Tennessee, Knoxville
**TRACE: Tennessee Research and Creative
Exchange**

Chancellor's Honors Program Projects

Supervised Undergraduate Student Research
and Creative Work

Spring 5-2007

Gene Expression in *Atp10c* +/- Mice

Helen Elizabeth Reaves
University of Tennessee - Knoxville

Follow this and additional works at: https://trace.tennessee.edu/utk_chanhonoproj

Recommended Citation

Reaves, Helen Elizabeth, "Gene Expression in *Atp10c* +/- Mice" (2007). *Chancellor's Honors Program Projects*.

https://trace.tennessee.edu/utk_chanhonoproj/1105

This is brought to you for free and open access by the Supervised Undergraduate Student Research and Creative Work at TRACE: Tennessee Research and Creative Exchange. It has been accepted for inclusion in Chancellor's Honors Program Projects by an authorized administrator of TRACE: Tennessee Research and Creative Exchange. For more information, please contact trace@utk.edu.

Gene Expression in *Atp10c* ^{-/+} Mice

Helen Reaves, Joshua Yuan, Lora Abbott, Sarah Elliott, Dr. Madhu Dhar
University of Tennessee College of Veterinary Medicine, Knoxville, TN

Abstract:

Diabetes is a worldwide health crisis of increasing prevalence and thus, of increasing concern. In this experiment, we are working with a novel model of type 2 diabetes, *Atp10c* ^{-/+} mice. *Atp10c* is an ATPase and a suspected aminophospholipid transporter, and loss of *Atp10c* results in the development of obesity and diabetes in mice on a high fat diet. The aims of this study are to elucidate the role of *Atp10c* in the insulin signaling pathway and thus, hopefully provide a novel target for diabetes therapy. In order to determine the role of *Atp10c*, this study examined the expression of several genes known to be involved in insulin-stimulated glucose transport in peripheral tissues. Focused pathway gene microarrays were used to narrow down the specific genes affected by loss of *Atp10c*, and real time polymerase chain reaction (PCR) was then used to confirm the gene microarray results for five important genes: *IRS1* (*insulin receptor substrate-1*), *PI3K p85 α* (regulatory subunit of *phosphoinositide 3-kinase*), *PDK1* (*phosphoinositide dependent kinase 1*), *PKB* (*protein kinase B/Akt2*), and *PKC λ /t* (isoform of *protein kinase C*). While much data was gathered in this study, the exact role of *Atp10c* on the expression of these five genes in the soleus muscle and gonadal fat is still unclear. Future studies to analyze post transcriptional and translational changes are needed to determine the biological role of *Atp10c* in glucose homeostasis.

Introduction:

Diabetes mellitus is an international health crisis, affecting 171 million people worldwide, and that number is expected to swell to 366 million by 2030.¹ In the United States,

7% of the United States population or 20.8 million people have diabetes, and if trends continue, nearly 1/3 of people born in 2000 will have diabetes in their lifetime.²

There are two types of diabetes: type 1 and type 2. Of all diagnosed diabetes cases, 90-95% are type 2 diabetes.² Factors that can predispose a person to develop type 2 diabetes include genetics, obesity, and a sedentary lifestyle. In this disease, cells develop insulin resistance, and in an attempt to compensate, the beta cells of the pancreas produce excess insulin. The beta cells are unable to produce additional insulin for an extended period of time and quit functioning. This leads to a decrease in insulin. The liver then responds to this decrease in insulin by producing excess glucose.^{2,3} Thus, the whole body is affected by type 2 diabetes.

In this project, we focused on a particular strain of mouse that develops type 2 diabetes and obesity when fed a high fat diet. These mice were developed by the Oak Ridge National Laboratory and harbor the deletion $p^{23DFiOD}$. Mice inheriting this deletion maternally have a 1.5 to 2 times increase in the sizes of individual fat pads; an increase in their adiposity index; an increase in kidney, liver, and spleen weights; hyperinsulinemia; altered insulin stimulated response in peripheral tissues; and insulin resistance.⁴ This deletion codes for three genes: *Atp10c*, *Gabrb3*, and *Ube3a*.⁴ The diabetic changes are associated with the loss of *Atp10c* but not with the loss of *Gabrb3* or *Ube3a*.⁴ Thus, *Atp10c* is believed to be the gene responsible for the diabetic changes in these mice, and these mice will be referred to as *Atp10c* $-/+$ mice in this paper.

Atp10c has been shown to belong to class IV of P-type ATPases, and it is believed to be an aminophospholipid translocase.^{4,5} Previous research has suggested that loss of *Atp10c* affects the normal translocation and sequestration of *GLUT4* in skeletal muscle and adipose tissue.⁶ The

purpose of this study is to characterize the changes that occur on the gene expression level due to loss of *Atp10c* in skeletal muscle and adipose tissue.

Materials and Methods:

Control and mutant groups: The mice used in the experiment are female *Atp10c* $-/+$ mice.

These mice undergo genetic imprinting such that those mice that inherit the deletion from their dam develop diabetic changes while those mice that inherit the deletion from their sire resemble wild type mice.⁴ Thus, the mice that inherit the deletion paternally have been designated as the control group, and the mice that inherit the deletion maternally have been designated as the mutant group.

The mice: The mouse line was maintained as described in *Physiological Genomics*.⁸ The mice were fed a regular chow diet until weaning at 4-5 weeks of age. They were then fed a high fat diet for 4 or 12 weeks. The procedure is described in *The Journal of Nutritional Biochemistry*.⁶ The time points chosen were based on earlier results.⁴ After the designated time period, they were euthanized via CO₂ asphyxiation, and the soleus muscle and gonadal fat were collected from each mouse. To provide sufficient tissue, the tissue from several mice were combined.

RNA extraction: RNA was extracted from the combined tissue using the Trizol method (Invitrogen Carlsbad, CA, USA).

RNA quantification: After the RNA was extracted from the tissue, an Eppendorf Biophotometer was used to quantitate the RNA. The RNA was diluted to a concentration of 2 μ L of RNA to 98 μ L of DEPC water and then quantitated according to the manufacturer's instructions.

Focused pathway gene microarray: Focused pathway gene microarrays were run on the soleus muscle from the 4 week and 12 week control groups and on the soleus muscle from the 4 week

and 12 week mutant groups. Mouse *Gapdh* was used as the control gene. The procedure used is described in *The Journal of Nutritional Biochemistry*.⁶

Determination of genes of interest: The focused pathway gene microarrays were used to narrow down the specific genes affected by loss of *Atp10c*. The gene expression between the mutant and control groups was considered significant if the ratio of gene expression between the mutant and control groups was $\geq 2.0:1$ or $\leq 0.5:1$. Previous research has suggested that loss of *Atp10c* somehow affects the normal translocation and sequestration of *GLUT4* in skeletal muscle and adipose tissue.⁶ Thus, several studies were examined to determine important genes in this pathway.^{9,10,11} These studies and the microarray results were then used to select five genes to analyze. The genes determined to be of interest were *IRS1* (*insulin receptor substrate-1*), *PI3K p85 α* (regulatory subunit of *phosphoinositide 3-kinase*), *PDK1* (*phosphoinositide dependent kinase 1*), *PKB* (*protein kinase B/Akt2*), and *PKC $\lambda/1$* (isoform of *protein kinase C*).

Primers: For each gene of interest, the National Center for Biotechnology Information website (<http://www.ncbi.nlm.nih.gov/>) was used to determine the nucleotide sequence. Then, Applied Biosystems's Primer Express® software was used to design appropriate primers for each gene, and each primer was ordered from Operon Biotechnologies.

DNA synthesis: Then, Bio-Rad's iScript™ cDNA Synthesis Kit was used to synthesize cDNA. Using the concentration of RNA calculated by the biophotometer, the amount of μL needed for 1 μg of RNA was calculated. Then, 2 μL of 5X iScript Reaction Mix, 0.5 μL of iScript Reaction Mix, 1 μg of RNA, and several μL of nuclease free water was added to a polymerase chain reaction (PCR) tube. Enough nuclease free water was added to each tube such that the total volume equaled 10 μL . Finally, the sample was placed into Sanyo's TaKaRa PCR Thermal

Cycler and set to run at 25°C for 5 minutes, 42°C for 30 minutes, 85°C for 5 minutes, and 4°C until the sample was removed from the machine.

Real time polymerase chain reaction (PCR): The synthesized cDNA was then amplified using the gene specific primers according to the procedure described in *The Journal of Nutritional Biochemistry*.⁶ Mouse *Gapdh* or mouse *18s* was used as the control gene.

Data analysis: The data was analyzed using the procedure and statistical analysis software (SAS) program described in *BMC Bioinformatics*.⁷

Results:

Focused pathway gene microarray data run on soleus muscle of the mice fed the high fat diet for 4 weeks showed a ratio of gene expression between the mutant and control mice to be 1.1:1 for *IRS-1*, 0.6:1 for *PI3K p85 alpha*, 1.7:1 for *Pdk1*, 1.1:1 for *Akt2*, and 1.4:1 for *PKC iota* (Table 1). On the other hand, the gene microarray run on the soleus muscle of the mice fed a high fat diet for 12 weeks showed a ratio of gene expression between the mutant and control mice to be 4.0:1 for *IRS-1*, 4.6:1 for *PI3K p85 alpha*, 0.5:1 for *Pdk1*, 2.1:1 for *Akt2*, and 2.4:1 for *PKC iota* (Table 2).

Real time polymerase chain reaction (PCR) run on soleus muscle of the mice fed the high fat diet for 4 weeks showed a ratio of gene expression between the mutant and control mice to be 2.2:1 for *IRS-1*, 0.9:1 for *PI3K p85 alpha*, 1.3:1 for *Pdk1*, 0.5:1 for *Akt2*, and 1.0:1 for *PKC iota* (Table 3). The values for *IRS-1* and *Akt2* are statistically significant ($p \geq 0.05$). On the other hand, the real time PCR run on the soleus muscle of the mice fed a high fat diet for 12 weeks showed a ratio of gene expression between the mutant and control mice to 0.1:1 for *IRS-1*, 0.7:1 for *PI3K p85 alpha*, 1.9:1 for *Pdk1*, 0.3:1 for *Akt2*, and 1.2:1 for *PKC iota* (Table 5). The values for *IRS-1*, *Pdk1*, and *Akt2* are significant. When the 4 week soleus muscle control group's

control gene values are substituted for the 12 week soleus muscle control group's control gene values, those values become 4.9:1 for *IRS-1*, 92.4:1 for *PI3K p85 alpha*, 26.3:1 for *Pdk1*, 1.8:1 for *Akt2*, and 171.9:1 for *PKC iota* (Table 6). The values for *IRS-1*, *PI3K p85 alpha*, *Pdk1*, and *PKC iota* are statistically significant. Also, when mouse *I8s* is used as the control gene rather than mouse *Gapdh*, the values become 0.7:1 for *IRS-1*, 1.0:1 for *PI3K p85 alpha*, 1.2:1 for *Pdk1*, 2.5:1 for *Akt2*, and 1.2:1 for *PKC iota* (Table 7). Only the value for *IRS-1* is statistically significant.

Real time polymerase chain reaction (PCR) run on gonadal fat of the mice fed the high fat diet for 4 weeks showed a ratio of gene expression between the mutant and control mice to be 0.3:1 for *IRS-1*, 0.7:1 for *PI3K p85 alpha*, 0.8:1 for *Pdk1*, 1.5:1 for *Akt2*, and 1.1:1 for *PKC iota* (Table 4). The values for *IRS-1* and *PI3K p85 alpha* are statistically significant ($p \geq 0.05$). On the other hand, the PCR run on the gonadal fat of the mice fed a high fat diet for 12 weeks showed a ratio of gene expression between the mutant and control mice to be 1.5:1 for *IRS-1*, 1.0:1 for *PI3K p85 alpha*, 2.4:1 for *Pdk1*, 4.7:1 for *Akt2*, and 0.9:1 for *PKC iota* (Table 8). The values for *IRS-1*, *PI3K p85 alpha*, *Pdk1*, and *PKC iota* are statistically significant ($p \geq 0.05$).

Table 1: Focused pathway gene microarray data comparing the gene expression in the soleus muscle of mutant mice to the gene expression in the soleus muscle of control mice after the mutant and control mice were fed the high fat diet for 4 weeks

Gene studied	Ratio of gene expression in mutant mice:control mice
<i>IRS-1</i>	1.1 : 1
<i>PI3K p85 alpha</i>	0.6 : 1
<i>Pdk1</i>	1.7 : 1
<i>Akt2</i>	1.1 : 1
<i>PKC iota</i>	1.4 : 1

Table 2: Focused pathway gene microarray data comparing the gene expression in the soleus muscle of mutant mice to the gene expression in the soleus muscle of control mice after the mutant and control mice were fed the high fat diet for 12 weeks

Gene studied	Ratio of gene expression in mutant mice:control mice
<i>IRS-1</i>	4.0 : 1
<i>PI3K p85 alpha</i>	4.6 : 1
<i>Pdk1</i>	0.5 : 1
<i>Akt2</i>	2.1 : 1
<i>PKC iota</i>	2.4 : 1

Table 3: Real time polymerase chain reaction (PCR) data using mouse *Gapdh* as a control gene and comparing the gene expression in the soleus muscle of mutant mice to the gene expression in the soleus muscle of control mice after the mutant and control mice were fed the high fat diet for 4 weeks

Gene studied	$-\Delta\Delta Ct$	P value	Ratio of gene expression in mutant mice:control mice ($2^{-\Delta\Delta Ct}$)
<i>IRS-1</i>	1.1210	0.0012	2.2 : 1 *
<i>PI3K p85 alpha</i>	-0.09	0.5	0.9 : 1
<i>Pdk1</i>	0.3590	0.0954	1.3 : 1
<i>Akt2</i>	-1.1248	0.0154	0.5 : 1 *
<i>PKC iota</i>	-0.05295	0.5750	1.0 : 1

*= statistically significant ($p \leq 0.05$)

Table 4: Real time polymerase chain reaction (PCR) data using mouse *Gapdh* as a control gene and comparing the gene expression in the gonadal fat of mutant mice to the gene expression in the gonadal fat of control mice after the mutant and control mice were fed the high fat diet for 4 weeks

Gene studied	$-\Delta\Delta Ct$	P value	Ratio of gene expression in mutant mice:control mice ($2^{-\Delta\Delta Ct}$)
<i>IRS-1</i>	-1.9607	0.0025	0.3 : 1 *
<i>PI3K p85 alpha</i>	-0.6004	0.0101	0.7 : 1 *
<i>Pdk1</i>	-0.3103	0.1100	0.8 : 1
<i>Akt2</i>	0.6148	0.0977	1.5 : 1
<i>PKC iota</i>	0.1366	0.4326	1.1 : 1

*= statistically significant ($p \leq 0.05$)

Table 5: Real time polymerase chain reaction (PCR) data using mouse *Gapdh* as a control gene and comparing the gene expression in the soleus muscle of mutant mice to the gene expression in the soleus muscle of control mice after the mutant and control mice were fed the high fat diet for 12 weeks

Gene studied	$-\Delta\Delta Ct$	P value	Ratio of gene expression in mutant mice:control mice ($2^{-\Delta\Delta Ct}$)
<i>IRS-1</i>	-3.3	0.0001	0.1:1*
<i>PI3K p85 alpha</i>	-0.6	0.13	0.7:1
<i>Pdk1</i>	0.9	0.03	1.9:1*
<i>Akt2</i>	-2.0	0.001	0.3:1*
<i>PKC iota</i>	0.3	0.6	1.2:1

*= statistically significant ($p \leq 0.05$)

Table 6: Real time polymerase chain reaction (PCR) data, using mouse *Gapdh* as a control gene and using 4 week soleus muscle control gene values, comparing the gene expression in the soleus muscle of mutant mice to the gene expression in the soleus muscle of control mice after the mutant and control mice were fed the high fat diet for 12 weeks

Gene studied	$-\Delta\Delta Ct$	P value	Ratio of gene expression in mutant mice:control mice ($2^{-\Delta\Delta Ct}$)
<i>IRS-1</i>	2.2950	<0.0001	4.9 : 1 *
<i>PI3K p85 alpha</i>	6.5298	<0.0001	92.4 : 1 *
<i>Pdk1</i>	4.7164	<0.0001	26.3 : 1 *
<i>Akt2</i>	0.8252	0.4172	1.8 : 1
<i>PKC iota</i>	7.4258	<0.0001	171.9 : 1 *

*= statistically significant ($p \leq 0.05$)

Table 7: Real time polymerase chain reaction (PCR) data, using mouse *I8s* as a control gene, comparing the gene expression in the soleus muscle of mutant mice to the gene expression in the soleus muscle of control mice after the mutant and control mice were fed the high fat diet for 12 weeks

Gene studied	$-\Delta\Delta Ct$	P value	Ratio of gene expression in mutant mice:control mice ($2^{-\Delta\Delta Ct}$)
<i>IRS-1</i>	-0.5567	>0.0001	0.7:1*
<i>PI3K p85 alpha</i>	-0.06653	0.5656	1.0:1
<i>Pdk1</i>	0.2743	0.2623	1.2:1
<i>Akt2</i>	1.3211	0.1156	2.5:1
<i>PKC iota</i>	0.2027	0.1777	1.2:1

*= statistically significant ($p \leq 0.05$)

Table 8: Real time polymerase chain reaction (PCR) data using mouse *Gapdh* as a control gene and comparing the gene expression in the gonadal fat of mutant mice to the gene expression in the gonadal fat of control mice after the mutant and control mice were fed the high fat diet for 12 weeks

Gene studied	$-\Delta\Delta Ct$	P value	Ratio of gene expression in mutant mice:control mice ($2^{-\Delta\Delta Ct}$)
<i>IRS-1</i>	0.6139	0.2864	1.5 : 1
<i>PI3K p85 alpha</i>	0.01603	0.9782	1.0 : 1
<i>Pdk1</i>	1.2873	0.0392	2.4 : 1 *
<i>Akt2</i>	2.2389	0.0838	4.7 : 1
<i>PKC iota</i>	-0.1964	0.7064	0.9 : 1

*= statistically significant ($p \leq 0.05$)

Discussion:

The focused pathway gene microarray run on the soleus muscle of the mice fed the high fat diet for 4 weeks showed the gene expression of *IRS-1*, *PI3K p85 alpha*, *Pdk1*, *Akt2*, and *PKC iota* to be approximately the same between the mutant and control groups. However, the gene microarray run on the soleus muscle of the mice fed the high fat diet for 12 weeks showed the gene expression of *IRS-1*, *PI3K p85 alpha*, *Akt2*, and *PKC iota* to be increased in the mutant group, and the gene expression of *PI3K p85 alpha* to be decreased in the mutant group.

The real time PCR run on the soleus muscle of the mice fed the high fat diet for 4 weeks showed the gene expression of *PI3K p85 alpha*, *Pdk1*, and *PKC iota* to be approximately the same between the mutant and control groups. The gene expression of *IRS-1* is increased (2.2:1) in the mutant mice, and the gene expression of *Akt2* is decreased (0.5:1) in the mutant mice.

The real time PCR run on the soleus muscle of the mice fed the high fat diet for 12 weeks presented problems, and thus, several experiments were performed. When mouse *Gapdh* was used as the control gene, the gene expression of *PI3K p85 alpha* and *PKC iota* was similar between the mutant and control groups. However, the gene expression of *Pdk1* was increased (1.9:1) in the mutant mice, and the gene expression of *IRS-1* and *Akt2* were decreased (0.1:1 and

0.3:1, respectively) in the mutant mice. On examining the values between the control group's *Gapdh* values and the mutant group's *Gapdh* values, it was noted that there was a large difference between the values. Since *Gapdh* was used as the control gene, its value should have been approximately the same in the control mice and the mutant mice. The 4 week soleus muscle *Gapdh* values were similar between the control and mutant groups. Thus, these values were substituted. This showed an increase in gene expression of all of the genes (*IRS-1*, *PI3K p85 alpha*, *Pdk1*, *Akt2*, and *PKC iota*) although the increase was not statistically significant for *Akt2*. In order to determine the actual gene expression, mouse *18s* was used as the control gene. This showed similar gene expression for *PI3K p85 alpha*, *Pdk1*, *Akt2*, and *PKC iota* in the control and mutant groups. However, the gene expression of *IRS-1* was decreased (0.7:1)

The real time PCR run on the gonadal fat of the mice fed the high fat diet for 4 weeks showed the gene expression of *Pdk1*, *Akt2*, and *PKC iota* to be approximately the same between the mutant and control groups. However, the gene expression of *IRS-1* and *PI3K p85 alpha* are decreased (0.3:1 and 0.7:1, respectively) in the mutant mice. The real time PCR run on the gonadal fat of the mice fed the high fat diet for 12 weeks showed the gene expression of *IRS-1*, *PI3K p85 alpha*, and *PKC iota* to be approximately the same between the mutant and control groups. The gene expression of *Pdk1* is increased (2.4:1) in the mutant mice, and while not statistically significant, the gene expression of *Akt2* is also increased (4.7:1) in the mutant mice.

While these results provide new data regarding the effects of *Atp10c* on gene expression of genes involved in the insulin signaling pathway, more research needs to be performed in order to make conclusions about *Atp10c*'s specific role. Of particular interest are the effects of *Atp10c* on protein expression and protein activity. While the expression of a particular gene may be increased or decreased, that does not dictate whether the protein coded for by that gene is present

or whether that protein is functional. Thus, more research is needed in order to determine how these changes in gene expression affect glucose homeostasis.

References:

- ¹*World Health Organization* (2006). 17 July 2006. <<http://www.who.int>>.
- ²American Diabetes Association. 17 July 2006. <<http://www.diabetes.org/>>.
- ³Leahy, Jack L. "Pathogenesis of Type 2 Diabetes Mellitus." *Archives of Medical Research*. 2005 May-Jun; 36 (3): 197-209.
- ⁴Dhar, MS.; Somvardahl, CS.; Kirkland, T.; et. al. "Mice Heterozygous for *Atp10c*, a Putative Amphipath, Represent a Novel Model of Obesity and Type 2 Diabetes." *The Journal of Nutrition*. 2004 Apr; 134 (4): 799-805. 27 Nov. 2006. <<http://jn.nutrition.org/cgi/content/full/134/4/799>>.
- ⁵Dhar, M.; Hauser, L; and Johnson, D. "An Aminophospholipid Translocase Associated with Body Fat and Type 2 Diabetes Phenotypes." *Obesity Research*. 2002 Jul; 10(7): 695-702. 27 Nov. 2006. <<http://www.obesityresearch.org/cgi/content/full/10/7/695>>.
- ⁶Dhar, MS.; Yuan, JS.; Elliott, SB; et. al. "A Type IV P-type ATPase Affects Insulin-Mediated Glucose Uptake in Adipose Tissue and Skeletal Muscle in Mice." *The Journal of Nutritional Biochemistry*. 2006 Dec; 17 (12): 811-20. 27 Nov. 2006. <<http://www.jnutbio.com/article/PIIS0955286306000209/abstract>>.
- ⁷Yuan, Joshua S.; Reed, Ann; Chen, Fend; et. al. "Statistical Analysis of Real-time PCR Data." *BMC Bioinformatics*. 2006 Feb 22; 7: 85.

- ⁹Krook, A; Wallberg-Henriksson, H; and JR. Zierath. "Sending the Signal: Molecular Mechanisms Regulating Glucose Uptake." *Medicine and Science in Sports and Exercise*. 2004 Jul; 36(7): 1212-7.
- ¹⁰Watson, R.T.; Kanzaki, M; and JE. Pessin. "Regulated Membrane Trafficking of the Insulin-Responsive Glucose Transporter 4 in Adipocytes." *Endocrine Reviews*. 2004 Apr; 25 (2): 177-204.
- ¹¹Chang, L; Chiang, SH; and AR Saltiel. "Insulin Signaling and the Regulation of Glucose Transport." *Molecular Medicine*. 2004 Jul-Dec; 10 (7-12): 65-71.