Pak. J. Agri. Sci., Vol. 55(3), 603-609; 2018
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
DOI: 10.21162/PAKJAS/18.6449
http://www.pakjas.com.pk

THE NICOTINAMIDE NUCLEOTIDE TRANSHYDROGENASE GENE COULD IMPAIR BLOOD GLUCOSE LEVEL STABILITY AND INCREASE BASAL METABOLISM

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C57BL/6J (B6J) is the most widely used mouse strain for metabolic research. It carries a spontaneous mutation in the nicotinamide nucleotide transhydrogenase (*Nnt*) gene. This study compared blood glucose levels in B6J and Kunming (Km) mice as control. It was observed that blood glucose levels in B6J and Km mice at 4 weeks of age decreased post feeding and reached the lowest level at 24 hr of fasting. Blood glucose was significantly higher in B6J mice than that in Km mice at 0 hr, 2 hr and 10 hr of fasting. In addition, the correlation between the *Nnt* gene, growth traits and the feed conversion efficiency ratio (FCR) in (B6J × Km) F2 generation (N = 342) was also analyzed. We found that *Nnt* was significantly associated with body weight at 3 weeks (IBW) ($P<0.01$) and 5 weeks (FBW) ($P<0.05$) and average metabolic body weight (AMBW) ($P<0.01$) but was not associated with average daily feed intake (AFI), average daily gain (ADG) and FCR. Furthermore, qRT-PCR revealed that the expression levels of *Glut-1*, *Glut-2*, *Akt-1*, *Irs-1* and *Ucp-2* genes were significantly different in high and low-FCR mice. Our study offers novel evidence of the roles of *Nnt* gene in metabolism and growth.
Keywords: *Nnt*, blood glucose, familial glucocorticoid deficiency, glucose metabolism, autosomal recessive disorder, feed conversion ratio, mice

INTRODUCTION

Nicotinamide nucleotide transhydrogenase is located at the inner membrane of the mitochondria and catalyses the reversible reduction of $NADP^+$ by NADH (Adedeji, 2009; Kelsey *et al.*, 2015) which is important for integrating the mitochondrial tricarboxylic acid cycle (TCA) cycle and energy metabolism (Natascia *et al.*, 2006; Fei *et al.*, 2012; Andrikopoulos, 2010). Several studies have indicated that the *Nnt* gene is significantly associated with human disease such as obesity (Toye *et al.*, 2005; John *et al.*, 2013) and Type 2 Diabetes (T2D) (Helen *et al.*, 2006; Grace *et al.*, 2014; Sadie and Nair, 2010). While, a few studies recently have reported that *Nnt* gene is related to familial glucocorticoid deficiency (FGD) which is a rare autosomal recessive disorder (Yasuko *et al.*, 2015; Juliana *et al.*, 2013; Eirini *et al.*, 2012).

Previously, several studies have also been proposed that *Nnt* gene could participate in glucose metabolism (Helen *et al.*, 2006). A five exon deletion of *Nnt* gene in B6J mice, which exhibits impaired glucose tolerance (IGT) and reduced insulin secretion, has been identified (Sofianos *et al.*, 2005). Furthermore, QTL mapping results indicated that the *Nnt* gene could be a candidate gene for glucose metabolism (Brand *et al.*, 2010). However, other studies indicate that mutating *Nnt* is associated with impaired β-cell mitochondrial metabolism, possibly *via* activation of uncoupling proteins, which may regulate glucose-stimulated insulin secretion (GSIS), energy balance, body weight and thermoregulation (Neil *et al.*, 2010; Adedeji *et al.*, 2009; Jessica and Carter-Su, 1988).

Most cells utilize glucose as their primary fuel source for energy (Jonhan *et al.*, 2012). Glucose uptake typically is mediated by a family of facilitative glucose transporters (GLUTs 1-4) that is differently regulated, reflecting their specific roles in cellular and whole body glucose homeostasis (Luc, 2013; Douglas *et al.* and Fan, 2009). It has been reported that glucose metabolism is related to growth (Anthony *et al.*, 2010). Yamada and co-workers study indicates that the fasting levels of glucose were negatively correlated with plasma growth hormone (GH) levels (Yamada *et al.*, 2010) which can significantly stimulate the

glucose oxidation (Møller *et al.*, 1990). Moreover, insulin-like growth factor I and II (IGF-I/II) could also stimulate an increase in glucose oxidation, with a longer and stronger effect than GH (Philip *et al.*, 1997). Further, thymoma viral proto-oncogene 1 (*Akt-1*) plays an important role in mediating glucose metabolism and protein synthesis (Bhanu *et al.*, 2010). Furthermore, *Akt-1* has a distinct role in glucose metabolism, maintaining insulin sensitivity and modulating the expression of the insulin receptor substrate-1 expression (*Irs-1*) gene (Hana *et al.*, 2012).

It has been reported that energy metabolism is related to feed efficiency (FE), which is a complex trait in animals (McDonald *et al.*, 2009). FE can be measured by a feed conversion ratio (FCR) or residual feed intake (RFI) (Chen *et al.*, 2011). Our previous study indicated that mitochondrial energy metabolism was negatively related to FE in pigs (Lu *et al*., 2015). Another study indicated that carbohydrate metabolism has also been related to FE in cattle (Chen *et al.*, 2011). Although the *Nnt* gene plays a role in glucose metabolism, the role of *Nnt* in FE is unclear. To investigate the role of *Nnt* gene in glucose metabolism, the blood glucose level of Km mice to normal *Nnt* and B6J mice with a mutant *Nnt* were compared. Moreover, the blood glucose levels between F2 generations (B6J × Km) of mice having different *Nnt* genotypes were also evaluated. Further, the roles of *Nnt* gene in growth and FE were detected by trait association studies in F2 generation mice. Furthermore, expression analysis of the genes related to glucose metabolism in liver tissues was also performed.

MATERIALS AND METHODS

***Experimental animals*:** B6J and Km mice were obtained from the Wuhan University Center for Animal Experiments and the Hubei Centre for Disease Control and Prevention at 3-5 weeks of age. Mice were housed under controlled temperature (21±2°C) on a 12:12 hour light- dark cycle with free access to food and water. For F2 generation, seven Km males were crossed with seven B6J females to generate 84 F1 mice which were crossed to generate F2 mice. Then, 342 F2 generation mice were used for the association study of *Nnt* gene. All the mice were fed a standard diet. The feed intake of the F2 mice at 3 to 5 weeks of age was measured. Additionally, the body weight at 3 and 5 weeks of age was measured (IBW and FBW). Furthermore, ADG, AMBW and FCR were measured as follows:

ADG = (FBW- IBW)/14; AMBW = $[(IBW+FBW)/2]^{0.75}$; FCR = AFI/ADG. All the methods in this study were carried out in accordance with the approved guidelines from the Regulation of the Standing Committee of Hubei People's Congress. Furthermore, all experimental protocols were approved by the Ethics Committee of Huazhong Agricultural University (HZAUMU2013-0005).

***Blood glucose measurement*:** The blood glucose level was detected using a blood glucose monitor (Andon, Tianjin, China). For blood glucose level detection, 11 B6J and 11 Km mice at four weeks of age were selected randomly. In addition, 40 mice of the F2 generation at five weeks of age with extremely high and low-FCR were selected for blood glucose level detection. All the mice used for blood glucose level detection were fasted overnight (12 hours), and then fed ad libitum for one hour. Subsequently, the blood glucose levels were detected at 2 hr, 6 hr, 10 hr, 14 hr and 24 hr of fasting.

***RNA extraction and cDNA synthesis*:** The total RNA was extracted from liver tissues with Trizol reagent (Invitrogen, Carlsbad, CA, USA) and treated with DNase I (Fermentas, Ottawa, ON, Canada). The concentration and quality of the RNA was assessed using NanoDrop 2000 (Thermo, Waltham, MA, USA). Reverse transcription was performed using the RevertAid First Strand cDNA Synthesis Kit (Fermentas, Thermo Fisher Scientific Inc.).

***PCR and Quantitative Real Time- PCR (qRT- PCR) assays*:** To identify the *Nnt* mutation, we designed two pairs of primers for cDNA amplification. The first primers (*Nnt*-D1) could amplify cDNA of both the mutant and the normal *Nnt* gene; the second primers (*Nnt*-D2) could only amplify the normal gene. All the sequences of the primers used in this study are listed in Table 1. For genotyping of the F2 generation, two pairs of primers were used. The first pair of primers (*Nnt*-Km) could only amplify *Nnt* DNA of Km mice (Stefanie *et al.*, 2012); the second pair of primers (*Nnt*-B6J) could only amplify *Nnt* DNA of B6J mice.

The expression levels of *Glut-1*, *Glut-2*, *Glut-4*, *Ucp-2*, *Akt-1*, *Irs-1* and *Igf-1* were detected by qRT- PCR and the primers are also listed in Supplementary Table 1. The qRT-PCR was performed by using iTaq™ Universal SYBR® Green Supermix (Bio Rad) and the CFX96 machine (California, USA).

***Statistical analysis*:** For the association study, we used the SAS 8.1 statistical package to perform a least- squares analysis (Russell, 1997). The model adopted is as follows:

$Y=X\beta + Z\Gamma + \varepsilon$, where Y is the phenotypic trait value for IBW, FBW, AFI, ADG, AMBW and FCR, respectively; $X\beta$ is the genotype effect of *Nnt* gene. AA, AB and BB indicate to *Nnt* genotypes *Nnt* −/−, *Nnt* +/− and *Nnt* +/+, respectively. $Z\Gamma$ is the random effect of gender; and ε is the random error. For the difference analysis of blood glucose level and gene expression, we used Student's T- test (two- tailed distribution, two samples and unequal variances) or F- test (one- way ANOVA) and the levels of statistical significance were determined at $P < 0.05$ and $P < 0.01$.

RESULTS

Table 1. The sequence of primers used.

Target Gene	Primer sequence (5`- 3`)	Annealing temperature (°C)	PCR product length (bp)
Nnt-D1	F- AGCTCAGGGCTATGATGCTC	62°C	B6J: 435 bp
	R- TCTGGAGGGTCCGTGGGTC		Km: 1189 bp
Nnt-D2	F- CTTGGTGCTGAACCCTTGGA	60°C	B6J: – bp
	R- TGACCCTTCCTTCATGGACTC		Km: 216 bp
GAPDH	F- GGGTGTGAACCACGAGAAAT	60°C	200 bp
	R- CCTTCCACAATGCCAAAGTT		
Nnt-Km	F- GGGCATAGGAAGCAAATACCAAGTTG	59°C	549 bp
	R- GTAGGGCCAACTGTTTCTGCATGA		
Nnt-B6J	F- GCCAGAAGAGTTTATCATTCAGAGG	60°C	522 bp
	R- GTATCAACAGTTACCTTGGACCCAC		
Glut-1	F- CTACAATCAAACATGGAACCACCGC	58°C	175 bp
	R- TTGGAGAAGCCCATAAGCACAGCAG		
Glut-2	F- GGCTTTCACTGTCTTCACTGCTGTA	61°C	131 bp
	R- ACGTCATAGTTAATGGCAGCTTTCC		
Glut-4	F- GTCCAATGTCCTTGCTCCAGCTCCT	60°C	132 bp
	R- TATGGTGGCGTAGGCTGGCTGTCCC		
Ucp-2	F- GGAGGTAGCAGGAAATCAGA	61°C	190 bp
	R- TAGGATGGTACCCAGAACGC		
Akt-1	F- TACTTCCTCCTCAAGAACGATGGCA	61°C	160 bp
	R- ACATGGAAGGTGCGCTCAATGACTG		
Irs-1	F- AGAAGCAGCCAGAGGATCGTCAATA	60°C	120 bp
	R- ATCGTACCATCTACTGAAGAGGAAG		
Igf-1	F- CCAACTCAATTATTTAAGATCTGCC	60°C	141 bp
	R- TCCACACACGAACTGAAGAGCATCC		
Tubulin	F- GACTATGGACTCCGTTCGCTC	60°C	150 bp
	R- TATTCTTCCCGGATCTTGCTG		

***Nnt mutation detection*:** To confirm the deletion mutation of the *Nnt* gene, two pairs of primers were used for PCR. The first pairs of primers generated a 435 bp PCR product in the B6J mice and a 1189 bp PCR product in the Km mice (Fig. 1a, b). The second pairs of primers were designed from exon 6 to exon 7 so that only the normal *Nnt* could be amplified. Using these primers, there was a 216 bp product in the Km mice and no PCR product in the B6J mice (Fig. 1c).

***Blood glucose levels were different between B6J and Km mice*:** To confirm the relationship between *Nnt* and glucose levels, we compared the blood glucose variation in Km and B6J mice in the fasting state. All mice were firstly fasted overnight (12 hours) and then fed one hour, which was defined as 0 hours of fasting. The glucose levels at 6 times points were detected; 0 hours, 2 hours, 6 hours, 10 hours, 14 hours and 24 hours of fasting of B6J and Km mice. The blood glucose levels declined regularly post feeding and reached the lowest level at 24 hr of fasting in both strains (Table 2).

Table 2. Blood glucose concentration (mmol/ L) at 4 weeks of age in the B6J and Km mice.

Fasting Time (hours)	B6J mean±SE (n = 11)	Km mean±SE (n = 11)	*P*-Value
0h	16.70±0.73	11.71±0.41	< 0.01
2h	13.06±0.47	10.97±0.36	0.02
6h	10.76±0.32	10.40±0.49	0.54
10h	10.37±0.62	8.75±0.26	0.03
14h	8.09±0.37	8.03±0.32	0.90
24h	4.72±0.09	6.95±0.28	< 0.01

Moreover, the blood glucose levels of B6J mice at fasting, 0 hr, 2 hr and 10 hr were significantly higher than in Km mice. At 24 hr of fasting, the blood glucose level was significantly higher in Km mice than in B6J mice. No significant

differences were observed at the other time points. Furthermore, we used F1 generation (B6J × Km) to construct F2 generation (N = 342). For the glucose detection, the extremely high (N = 20) and low (N = 20) FCR mice (P<0.01) were selected (Table 3). However, no significant differences were observed at 0 hr, 12 hr and 24 hr of fasting in male and female mice.

Association analysis of the Nnt with growth and FE traits: To analyse the role of *Nnt* in growth and feed efficiency traits, we studied the correlation between the *Nnt* gene and the growth and FE traits in our (B6J × Km) F2 generation.

For genotyping, we designed two specific pairs of primers for the amplification of the mutant *Nnt* of B6J mice and the normal *Nnt* of Km mice. We obtained a 549 bp fragment of the normal *Nnt* gene of Km mice (Fig. 2a), as well as a 522 bp fragment of the mutant *Nnt* gene of B6J mice (Fig. 2b). While, no significant difference was found between AB and AA or BB (Fig. 3).

Furthermore, the association analysis results indicated that the *Nnt* gene was significantly associated with IBW (P< 0.01), FBW (P<0.05) and AMBW (P<0.01) but was not significantly associated with AFI, ADG and FCR (Table 4).

These specific primers were then used for genotyping the F2 generation (Fig. 2c). On the other hand, the variation of the glucose level between 0 hr and 12 hr of fasting was detected in F2 mice (N = 30). We compared the difference among three genotypes of *Nnt*, which were named as AA (0-12), AB (0-12) and BB (0-12), respectively. The statistical results showed that there was significant difference between AA and BB genotypes (P< 0.05).

Moreover, the IBW, FBW and AMBW values for the AA (*Nnt* −/−) and AB (*Nnt* +/−) genotypes were significantly higher than for the BB (*Nnt* +/+) genotype (P< 0.05).

Table 3. Blood glucose concentration (mmol/ L) at 5 weeks of age in the F2-FCR mice.

Time		Female			Male	
(hours)	mean±SE (n=10)	mean±SE (n=10)	P- Value	mean±SE (n=10)	mean±SE (n=10)	P-Value
FCR	5.19±0.17	8.56 ± 0.31	< 0.01	5.45±0.15	7.21±0.34	< 0.01
0h	10.83±0.86	9.73 ± 0.78	0.36	13.38±0.97	11.87±0.95	0.28
12h	6.53±0.67	6.04 ± 0.66	0.61	8.93±0.80	7.85±0.90	0.38
24h	4.84±0.49	4.42 ± 0.71	0.63	4.55±0.29	5.03±0.59	0.48

***Expression of other glucose metabolism related genes in the liver tissue of the high and low-FCR mice*:** To further investigate the correlation between glucose metabolism and the FCR trait, we also detected the expression levels of *Glut-1*, *Glut-2*, *Glut-4*, *Ucp-2*, *Akt-1*, *Irs-1* and *Igf-1*. *Glut-1* and *Ucp-2* expression levels were higher in the high-FCR mice than in the low-FCR mice. The expression levels of *Akt-1*, *Glut-2* and *Irs-1* genes were higher in the low-FCR mice than

FE, we detected the expression genes related to glucose metabolism in the liver of the high and low-FCR mice. Because the *Nnt* gene was not significantly associated with in the high-FCR mice (Fig. 4a, b). There were no significant differences in *Glut-4* and *Igf-1* genes expression.

Table 4. Association analysis of *Nnt* genotypes with growth and FE traits.

Traits	Genotypes			P-Value
	AA±SE (n= 3)	AB±SE (n=174)	BB±SE (n=75)	
IBW (g)	10.58^{a} ±0.21	10.37^{a} ±0.15	9.36^{b} ±0.23	< 0.01
FBW (g)	21.07^{a} ±1.45	21.35^{a} ±1.42	19.67^{b} ±1.47	0.03
AFI （g/day）	4.25±0.25	4.37±0.24	4.30±0.25	0.41
ADG （g/day）	0.72±0.07	0.74±0.07	0.73±0.07	0.50
AMBW $(g)^{0.75}$	7.91^{a} ±0.29	7.92^{a} ±0.28	7.42^{b} ±0.30	< 0.01
FCR (g feed/g gain)	6.07±0.28	6.09±0.27	6.01±0.29	0.87

The data are presented as the Mean + SE. Superscript letters a, b indicate significant difference, P<0.05.

DISCUSSION

NNT is considered a high- capacity source of mitochondrial

NADPH, which is located at the inner mitochondrial membrane (Yu-Ting *et al.*, 2012). In this study, we first confirmed that there was a deletion mutant of *Nnt* in B6J mice of 754 bp in the exon. Some studies indicated that the mutant *Nnt* could affect glucose metabolism (Freeman *et al.*, 2006). However, another study reported that there was no correlation between *Nnt* and glucose metabolism (Josef *et al.*, 2002). Therefore, the role of *Nnt* in glucose metabolism is not clearly understood.

To study the role of the *Nnt* gene in glucose metabolism, we compared the blood glucose level variations in the *Nnt* mutant B6J mice and the *Nnt* normal Km mice. Also, we analyzed the blood glucose level variations in the (B6J × Km) F2 generation. According to our results, post feeding, the blood glucose level reached a very high level and then gradually declined until 24 hr of fasting. In addition, the blood glucose level of the B6J mice was higher than the Km mice at the early stage of fasting, whereas it was lower than that in the Km mice at 24 hr. These results indicate that the glucose level of the Km mice was metabolized more slowly than in B6J. Also, the blood glucose level variation of *Nnt*−/− genotype was significantly higher than that of *Nnt*+/+ genotype in the F2 generation. These results indicate that the *Nnt* gene could participate in the glucose metabolism and may maintain the stability of the glucose level.

Nnt also plays a critical role in energy metabolism. The ATP level was reduced when the *Nnt* gene was knocked down (Fei *et al.*, 2012). Many studies have suggested that energy metabolism is related to FE (Chen *et al.*, 2011). The role of *Nnt* in FE is not clear. In this study, we found that *Nnt* was associated with AMBW and body weight, but not with FE. AMBW is positively correlated with basal energy metabolism (Chan *et al.*, 2001; Stefano and Mammucari, 2011).

Further, to investigate the differences in the body weight (BW) and growth between B6 and Km mice, the body weights of mice were measured at 3 weeks and 5 weeks of age. We observed that the body weight and growth of B6J and Km mice were significant different at 5 weeks age ($P < 0.01$) (Fig. 5). While, there were no significant differences at 3 weeks of age ($p = 0.2691$). Moreover, a previous study confirmed that mice carrFeig a mutant *Nnt* gene grew faster when fed a high-fat diet (Emily *et al.*, 2013). Therefore, *Nnt* gene was associated with the body weight, indicating that *Nnt* gene may play a role in growth processes.

Importantly, the glucose transporter genes were significantly expressed higher in the high- RFI than that in the low- RFI in the livers of Angus cattle (Polyana *et al*., 2015). Further, one study has reported that the expression of glucose transporter genes is variant between distinct species, tissues and cellular subtypes and each has differential sensitivities to stimuli such as insulin (Sumera *et al.*, 2012). Our results showed that, the blood glucose level was not significantly different between the high and low-FCR mice. In addition, the *Glut-4* gene was not significantly different between the high and low-FCR mice. Although the *Glut-1* and *Glut-2* genes were significant, their expression patterns between high and low-FCR mice were the opposite. Therefore, our results are consistent with a previous study showing that the expression level of *Glut-1* gene was higher than that of *Glut-2* and *Glut-4* in the liver tissue of rats (Michael *et al*., 1991; Cedric *et al*., 2013). Based on these results, we demonstrated that the blood glucose level may not associate with FE in mice.

Additionally, the expression level of *Igf-1* gene was not significantly different between the high and low-FCR mice. Further, we found that the expression of *Akt-1* and *Irs-1* genes was significantly higher in the low-FCR mice. *Akt-1* and *Irs-1* genes are key genes of the IGF signaling pathway (Chan *et al.*, 2001), which plays an important role in muscle growth and glucose metabolism (Stefano and Mammucari, 2011; Neil and Johnston, 2010). Therefore, we concluded that muscle growth might positively relate to FE in mice. It has been reported that the expression of *Ucp-2* is positively related to energy expenditure (Jessica and Carter-Su, 1988). According our results, the *Ucp-2* was significantly higher in the high-FCR mice. Therefore, we deduced that energy expenditure was negatively related to FE in mice.

***Conclusion*:** The blood glucose level of mutant *Nnt* B6J mice was significantly different than normal *Nnt* Km mice. The blood glucose level of the Km mice was metabolized more slowly than in B6J mice under fasting conditions. Additionally, the *Nnt* gene was associated with body weight and AMBW, but was not associated with FE. The expression levels of the *Akt-1* and *Irs-1* genes were higher and *Ucp-2* gene expression was lower in the liver of the high-FCR mice. Therefore, the *Nnt* gene could play a role in glucose metabolism and basal energy metabolism in mice.

***Acknowledgments*:** This research was supported by grants from the National Natural Science Foundation of China (NSFC) (No. 31372291), the National Key Basic Research Program of China (No. 2012CB124702), the research fund for the Doctoral Program of Higher Education for priority area (20130146130001) and the Fundamental Research Funds for the Central Universities (No. 2013PY050).

REFERENCES

Adedeji, O.O. 2009. Interrelationship of growth hormone, glucose and lipid metabolism. Nig. Q. J. Hosp. Med. 19:155-157.

Andrikopoulos, S. 2010. Obesity and Type 2 diabetes: Slow down! – Can metabolic deceleration protect the islet beta cell from excess nutrient- induced damage? Mol. Cell. Endocrinol. 316:140-146.

Anthony, N., R.C. Peter, M. Rachel, L.A. Charlotte, M.R. Grant, Z. Weidong and L.H. Edward. 2010. Diet induced obesity in two C57BL/6 sub-strains with intact or mutant Nicotinamide Nucleotide Transhydrogenase (Nnt) gene. Obesity (Silver Spring) 18:1902-1905.

Bhanu, M.C, K. Salmaan, L. Alex and H. Wei-Shou. 2010. Glucose metabolism in mammalian cell culture: new insights for tweaking vintage pathways. Trends. Biotechnol. 28:476-484.

Brand, M.D., N. Parker, C. Affourtit, S.A. Mookerjee and V. Azzu. 2010. Mitochondrial uncoupling protein 2 in pancreatic β- cells. Diabetes. Obes. Metab. 12:134-140.

Cedric, J., N. Charles, G. Marie-Hélène, C. Sibel, M.J. Willy and S. Abdullah. 2013. Comparison of GLUT1, GLUT2, GLUT4 and SGLT1 mRNA Expression in the Salivary Glands and Six Other Organs of Control, Streptozotocin-Induced and Goto-Kakizaki Diabetic Rats. Cell Physiol. Biochem. 31:37-43.

Chan, T.W., M. Pollak and H. Huynh. 2001. Inhibition of insulin- like growth factor signaling pathways in mammary gland by pure antiestrogen ICI 182, 780. Clin. Cancer Res. 7:2545-2554.

Chen, Y., C. Gondro, K. Quinn, R.M. Herd, P.F. Parnell and B. Vanselow. 2011. Global gene expression profiling reveals genes expressed differentially in cattle with high and low residual feed intake. Anim. Genet. 42:475-90.

Douglas, W.C. and F. WeiWei . 2009. The pathophysiology of mitochondrial disease as modeled in the mouse. Genes. Dev. 23:1714-1736.

Eirini, M., K. Julia, G. Leonardo, H.R. Claire, W. Florian, F. Peter, N. Peter, M.P. Nicholas, B. Ritwik, S.H. Nurcin , C.J. Paul , K.J. Peter, C.J.L. Adrian and M.A. Louise. 2012. Mutations in Nicotinamide Nucleotide Transhydrogenase (NNT) cause familial glucocorticoid deficiency. Nat. Genet. 44:740-742.

Emily, S.K., H. Masayuki, M.L. David, T.A. Sarah, K. Tatsuyoshi, C.Z. Zunaira, D.H. Kathleen, M.R. Dan, S.D. Natalie, M.G. Raghavendra and E. Carmella. 2013. Divergent compensatory responses to high- fat diet between C57BL6/J and C57BLKS/J inbred mouse strains. Am. J. Physiol. Endocrinol. Metab. 305:E1495-E1511.

Fei, Y., S. Harsh and C. Enrique. 2012. Silencing of nicotinamide nucleotide transhydrogenase impairs cellular redox homeostasis and energy metabolism in pc12 cells. Biochim. Biophys. Acta. 1817:401-409.

Freeman, H., K. Shimomura, R.D. Cox and F.M. Ashcroft. 2006. Nicotinamide nucleotide transhydrogenase: a link between insulin secretion, glucose metabolism and oxidative stress. Biochem. Soc. Trans. 34:806-810.

Grace, F., É. Mélanie, G. Mélanie, C. Chloé, A. Camille, J. Erik, F. Xavier, P. Marc, P. Vincent and A. Thierry. 2014. Defective insulin secretory response to intravenous glucose in C57Bl/6J compared to C57Bl/6N mice. Mol. Metab. 3:848-854.

Hana, S., F. Betina, S. Rita and I. Joseph. 2012. The expression of the class 1 glucose transporter isoforms in human embryonic stem cells, and the potential use of GLUT2 as a marker for pancreatic progenitor enrichment. Stem. Cells Dev. 21:1653-1661.

Helen, F.C., H. Alison, D.T. Neil, A.M. Frances and C.D. Roger. 2006. Deletion of Nicotinamide Nucleotide Transhydrogenase: A new quantitative trait locus accounting for glucose intolerance in C57BL/6J Mice. Diabetes 55:2153-2156.

Jessica, S. and C. Christin. 1988. Effects of growth hormone on glucose metabolism and glucose transport in 3T3-F442A cells: dependence on cell differentiation. Endocrinology 122:2247-2256.

John, H.T., K. Matthias, K. Joanna, F. Gesine, S. Michael, S. Edward, L. Tobias, D. Miriam, K. Peter, B. Matthias and K. Nora. 2013. Nicotinamide nucleotide transhydrogenase mRNA expression is related to human obesity. Obesity (Silver Spring) 21:529-534.

Jonhan, H., D.B. Michelle, L. Yan, V. Garret, T. Stephen, D.M. Lyn, H. Lin, K.M. John, B. Dorothea, H.V. Bennett and M.J. Stergios. 2012. Importance of glycolysis and oxidative phosphorylation in advanced melanoma. Mol. Cancer 11:76.

Josef, S.V., G. Yaoting and M.J. Liam. 2002. Impaired glucose homeostasis in insulin- like growth factor-binding protein-3- transgenic mice. Am. J. Physiol. Endocrinol. Metab. 283:E937-E945.

Juliana, R.A., F.R. Tiago, R.G. Felipe, O.C.F. Helena, V.E. Anibal and C.F. Roger. 2013. A spontaneous mutation in the nicotinamide nucleotide transhydrogenase gene of C57BL/6J mice results in mitochondrial redox abnormalities. Free Radic. Biol. Med. 63:446-456.

Kelsey, F.H., L. Chien-Te, R.E. Terence, R.R. Lauren, G.A.A Laura, C.L. Brook, L.S. Daniel, S.D. Cody, M.M. Deborah and N.P. Darrell. 2015. Pyruvate dehydrogenase complex and nicotinamide nucleotide transhydrogenase constitute an energy- consuming redox circuit. Biochem. J. 467:271-280.

Lu, J., Hou Y., M. Yuanxin, L. Xinyun, C. Jianhua, B.M. John, P. Tim and Z. Shuhong. 2015. Transcriptome analysis of mRNA and miRNA in skeletal muscle indicates an important network for differential Residual Feed Intake in pigs. Sci Rep 5: 11953.

Luc, T., E. Leonie, L. Virgile and S. Pascal. 2013. Effects of fructose- containing caloric sweeteners on resting energy expenditure and energy efficiency: a review of human trials. Nutr. Metab. 10:1-10.

McDonald, J.M., J.J. Ramsey, J.L. Miner and M.K. Nielsen. 2009. Differences in mitochondrial efficiency between lines of mice divergently selected for heat loss. J. Anim. Sci. 87:3105-3113.

Michael, T., K.B. Barbara and L.F. Harvey. 1991. Expression of the low Km GLUT-1 glucose transporter is turned on in perivenous hepatocytes of insulin-deficient diabetic rats. Endocrinology 129:1933-1941.

Møller, N., J.O. Jørgensen, K.G. Alberti, A. Flyvbjerg and O. Schmitz. 1990. Short-term effects of growth hormone on fuel oxidation and regional substrate metabolism in normal man. J. Clin. Endocrinol. Metab. 70:1179-1186.

Natascia, V., R.L. Shane and T. Roberto. 2006. Long- lived *C. elegans* Mitochondrial mutants as a model for human mitochondrial- associated diseases. Exp. Gerontol. 41:974-991.

Neil, I.B. and J.A. Ian. 2010. Transcriptional regulation of the IGF signaling pathway by amino acids and insulin-like growth factors during myogenesis in Atlantic salmon. PLoS One 5:E11100.

Nicole, W., B.R. Amy, M. Grant and A. Sofianos. 2010. The deletion variant of Nicotinamide Nucleotide Transhydrogenase (Nnt) does not affect insulin secretion or glucose tolerance. Endocrinology 151:96-102.

Philip, C., A.R. Dario and C.A.E Darren. 1997. PDK1 one of the missing links in insulin signal transduction? FEBS Lett. 410:3-10.

Polyana, T.C., C.L. Luiz, D.E. Jared, S.D. Robert, R.O. Kamila, O.S.N. Priscila, S.M. Marcela, M.B. Gerson, T.R. Rymer, C.S. Amália, L.P.D. Dante, Z. Adhemar, M.A. Mauricio, T.F. Jeremy and R.C.A. Luciana. 2015. Global liver gene expression differences in Nelore steers with divergent residual feed intake phenotypes. BMC Genomics 16:242.

Russell, W.D. 1997. An example of using mixed models and PROC MIXED for longitudinal data. J. Biopharm. Stat. 7:481-500.

Sadie, H.L. and K.S. Nair. 2010. Protein and energy metabolism in type 1 diabetes. Clin. Nutr. 29:13-17.

Sofianos, A., M.M. Christine, A. Kathryn, F. Alexandra, F.C. Barbara, H.L. Rebecca, K.E. Steven and P. Joseph. 2005. Differential effect of inbred mouse strain (C57BL/6, DBA/2, 129T2) on insulin secretory function in response to a high fat diet. J. Endocrinol. 187:45-53.

Stefanie, N.J., T. Tuyen, L.A. Charlotte, R.J. Andrea, W.G. Katrina and M.R. Grant. 2012. The C57BL/6J mouse strain background modifies the effect of a mutation in Bcl2l2. G3: Genes Genomes Genetics 2:99-102.

Stefano, S. and M. Cristina. 2011. Regulation of skeletal muscle growth by the IGF1-Akt/PKB pathway: insights from genetic models. Skelet Muscle 1:1-14.

Sumera, K., A.H. David and L.F. Patricia. 2012. Hepatic expression and cellular distribution of the glucose transporter family. World J. Gastroenterol. 18:677-6781.

Toye, A.A., J.D. Lippiat, P. Proks, K. Shimomura, L. Bentley, A. Hugill, V. Mijat, M. Goldsworthy, L. Moir, A. Haynes, J. Quarterman, H.C. Freeman, F.M. Ashcroft and R.D. Cox. 2005. A genetic and physiological study of impaired glucose homeostasis control in C57BL/6J mice. Diabetologia 48:675-686.

Yamada, P.M., H.H. Mehta, D. Hwang, K.P. Roos, A.L. Hevener and K.W. Lee. 2010. Evidence of a role for insulin- like growth factor binding protein (IGFBP)-3 in metabolic regulation. Endocrinology 151:5741-5750.

Yasuko, F., N. Eleonora, W. Sarah, S. Gyu, Y. Rie, M. Toshiharu, N. Keisuke, O. Tsutomu and G. Cecilia. 2015. Impact of a novel homozygous mutation in nicotinamide nucleotide transhydrogenase on mitochondrial DNA integrity in a case of familial glucocorticoid deficiency. BBA Clinical 3:70-78.

Yu-Ting, l., C. Mei-ling, C.T. Daniel and H. Hung-yao. 2012. Involvement of Nicotinamide Nucleotide Transhydrogenase in Cell Growth Regulation. Free Radical Biol. Med. 53:S44.