

# The Relative Gene Expression of GAPDH in Mice Fed with a Short-Term High Fat Diet

Mariam DESSAY<sup>1, 2</sup>, Hasnaa MAKSOURI<sup>2</sup>, Myriam RIYAD<sup>2</sup>, Paolo AJMONE MARSAN<sup>3</sup>, Khadija AKARID<sup>1</sup>, Fatima CHEGDANI<sup>1</sup>

1: University Hassan II Casablanca, Faculty of sciences Ain Chock Casablanca, Morocco

2: University Hassan II Casablanca, Faculty of Medicine and Pharmacy, Casablanca, Morocco.

3: Nutrigenomics Centre Università Cattolica Del Sacro Cuore, Piacenza, Italy.

## ABSTRACT

The important role of nutrition in health and disease prevention is well recognized. The glyceraldehyde-3-dehydrogenase phosphate gene (GAPDH) is considered as a housekeeping gene: it encodes a key enzyme of the glycolysis, and seems to affect the metabolism of fatty acids. The purpose of this work was to study the relative expression of the GAPDH gene in mice subjected to a short-term high fat diet. The expression of the GAPDH gene has been analyzed in the livers of C57BL / 6N mice: 5 control mice and 5 mice subjected to a short term high-fat diet. The quantification of the mRNA has been performed by a real-time PCR using specific primers designated and validated *in silico*. The data have been analyzed by the REST software. The results showed a significant down expression ( $p < 0.005$ ) of the GAPDH gene in mice subjected to high-fat diet. This suggests that a lipid diet may have an effect on the expression of the GAPDH gene. Thus the use of GAPDH as a reference gene should be reconsidered notably in gene expression studies.

**Keywords:** GAPDH, high-fat Diet, Housekeeping Gene, Lipid Metabolism, Mice

## I. INTRODUCTION

Currently, the prevalence of health problems related to nutrition in humans like obesity and diabetes are increasing. This phenomenon is strongly associated with a sedentary lifestyle. Also, these problems may be the consequence of a gene expression variation, especially at constitutive genes commonly considered as housekeeping gene.

The housekeeping genes mainly code for proteins essential to basic cell functions, e.g. beta actin, tubulin alpha (cytoskeleton) and Beta microglobulin (Major Histocompatibility Complex type I). These genes are stably expressed in all cells and play an important role in the metabolism and homeostasis [1]. Among them, GAPDH (Glyceraldehyde-3-phosphate dehydrogenase) also represents a gene traditionally used as a reference in expression

studies [8]. In the liver, the glycerol 3-phosphate derived from the lipolysis is essentially converted to dihydroxyacetone phosphate (DHAP) by GAPDH before joining the glycolysis; which makes GAPDH a key enzyme in glycolysis and also in fatty acid metabolism [2]. Thomas. D *et al* (2000) demonstrates that expression of beta actin and GAPDH was affected in quantitative serum-stimulation studies [8].

Hence the aim of this preliminary work was to investigate the effect of a high-fat diet on the GAPDH gene expression in mice.

## II. METHODS AND MATERIAL

We used mice C57BL/6N 3 weeks old submitted or not to a high-fat diet as described previously [3]. The relative expression of the GAPDH gene has been analyzed in mice liver samples: 5 controls were

subjected to a normal diet, and 5 mice subjected to a high-fat diet (HF). The livers tissues were sampled and preserved at -80 °C until RNA extraction for gene extraction analysis by real time PCR (Applied Biosystems 7500) using Syber Green. The beta-glucuronidase (GUSB) and hypoxanthine phosphoribosyltransferase (HPRT) were used as reference genes [4].

The primers sequences (Table 1) were designed across consecutive exons using Primer3 software (<http://frodo.wi.mit.edu/>).

Table 1: Primers sequences

Gene name	Primer sequence	PCR Product Size	Tm
GAPDH	Fw: GGAGAAACCTGCCAAGTATG	100 pb	60°C
	Rev: AGGAGACAACCTGGTCCTCA		
GUSB	FW: CGAACCCAGTCACCGCTGAGA	100 pb	60°C
	Rev: CTTCCGAAACACTGGGTCT		
HPRT	FW: ATTATGGACAGGACTGAAGC	120 pb	60°C
	Rev: AGGAGACAACCTGGTCCTCA		

Legend: Tm: Annealing temperature

Table 2: Relative GAPDH gene expression after normalization

Gene	Type	Reaction Eff	F.d	Std Erro	C.I 95%	P(H1)	Result
GAPDH	TAG	1	0,270	0,059-1,401	0,015-1,401	0,005	Down

Legend:

TRG: Gene Target

F.D: Fold Change

C.I: Confidence Interval

P (H1): Probability of alternate hypothesis that difference between Sample and control groups is due only to chance.

Interpretation

GAPDH is DOWN-regulated in sample group (in comparison to control group) by a mean factor of 0,270 (S.E. range is 0,059 - 1,401).

GAPDH sample group is different to control group. P(H1)=0,005

Relative gene expression was calculated using the Data-Assist™ v2.7 beta software (Applied

Biosystems) and REST 2009 (V2.0.7; Corbett Research and Pfaffl; (Pfaffl et al. 2002)). For target gene, expression data was expressed as fold change between HF group and CTR group (Table 2). Differences were considered as significant at  $p < 0.05$ .

### III. RESULT AND DISCUSSION

Our results showed a significant down expression ( $p < 0.005$ ) in mice fed with a short-term high-fat diet (Table 2). Barroso *et al* (1999) showed also a variation of the GAPDH expression after insulin stimulation in adipose tissue [5]. However, Robert. D *et al* (2005) showed a constant GAPDH expression in 72 human tissues in various situations [6]. Thus, as recommended by Caradec J *et al* (2010) and Thomas D. *et al* (2000) the expression of any gene should be accurately and systematically evaluated before their use as a reference gene.

### IV. CONCLUSION

Our preliminary results suggested that a high fat diet might have an effect on GAPDH gene expression. If the further analysis on a larger sample confirms these results, thus the use of GAPDH as a housekeeping gene should be reconsidered

### V. REFERENCES

- [1] Janet. A (2003). Comparison of human adult and fetal expression and identification of 535 housekeeping/maintenance genes. Affymetrix, Inc. Santa Clara, California 95051
- [2] Henry. E (2015). Beyond Glycolysis: GAPDHs Are Multifunctional Enzymes Involved in Regulation of ROS, Autophagy, and Plant Immune Responses. PLOS Genetics; DOI: 10.1371/journal.pgen.1005199
- [3] Lizier. M(2013). The nutrigenomic investigation of C57BL/6N mice fed a short-term high-fat diet highlightsearly changes in clock genes expression. Genes Nutr (2013) 8:465–474
- [4] Livak. KJ, Schmittgen TD (2001). Analysis of relative gene expression data using real-time

quantitative PCR and the  $2^{-\Delta\Delta C_T}$  method. *Methods* 25:402–408

- [5] Barroso. I. (1999). Norepinephrine, triiodothyronine and insulin upregulate glyceraldehyde-3-phosphate dehydrogenase mRNA during brown adipocyte differentiation. *European Journal of Endocrinology*; 141:169-179.
- [6] Robert. D (2005). GAPDH as a housekeeping gene: analysis of GAPDH mRNA expression in a panel of 72 human tissues. *Physiol Genomics* 21: 389–395.
- [7] Caradec. J; Sirab. C (2010). Desperate house genes: the dramatic example of hypoxia. *British Journal of Cancer*; 102:1037–1043.
- [8] Thomas D.; Brain A. (2000). Effect of experimental treatment on housekeeping gene expression: validation by real-time, quantitative RT-PCR