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Quick and sensitive determination of gene expression of hormone-sensitive lipase by real-time PCR

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Abstract—**Background: Obesity results from an imbalance between energy intake and energy expenditure, which leads to a pathological accumulation of adipose tissue, but the underlying mechanism at gene level is far from being elucidated. Methods: Pig primary adipocytes were cultured in vitro and the expression of HSL was detected by real-time PCR. Results: The high concentrations of glucose group significantly cut down the mRNA expression of HSL (P<0.01) rather than in the others. Conclusion: Our results provide significant data for that confirm an association between different glucose level and HSL expression in preadipocytes.**

Keywords- Energy；Lipid deposition；Hormone-sensitive lipase；mRNA express; Real-time-PCR

I. INTRODUCTION

Excessive weight gain arises from the interactions among environmental factors (dietary intake and physical activity), genetic predisposition and the individual behaviours [1]. Thus, genetic predisposition is important determinants for the increasing prevalence of the metabolic syndrome and associated complications, namely obesity [2].

Obesity is strongly associated with the metabolic syndrome and related to many diseases, such as hypertension, heart disease, and diabetes. Obesity results were from an imbalance between energy intake and energy expenditure, which leads to a pathological accumulation of adipose tissue [3]. This development of adipose tissue is caused by hypertrophy and hyperplasia of adipocytes. Therefore, an understanding of the molecular basis of hyperplasia as well as hypertrophy would contribute to the establishment of medical treatments to prevent health risks that can cause serious illness and death [4].

One explanation for the increase in the number of adipocytes is that it uptake of exogenously derived fatty acids (FA) and *de novo* of biosynthesis of FA was increased and then hydrolyzing acylglycerols was decreased. Among the different mechanisms that could lead to fat deposition and obesity, the epigenetic regulation of gene expression has emerged in the last years as a potentially important contributor [1]. Recent studies have revealed that several genes, such as Hormone-sensitive Lipase (HSL), play important roles in the stages of adipocyte metabolism [5]. The events occurring during the stages of adipocyte metabolism are relatively well characterized, but the molecular mechanisms underlying the stages of adipogenesis remain unknown.

The aim of this study was to analyze the correlation between the energy level with HSL expression in primary adipocytes. The results of this study provide some interesting data that may be beneficial to understand the molecular mechanisms underlying the stages of adipogenesis remain.

II. MATERIALS AND METHODS

a) Isolation of adipocyte precursors from pig adipose tissue

Epididymal fat pads of healthy pig (aged 18–20 days, Landrace) were removed under sterile conditions and transferred to ice-cold DMEM/F12 medium containing penicillin, streptomycin and fungizone (Sangon Biological Engineering Technology &Services Co., Ltd, Shanghai, China). Preadipocytes were isolated as described by Jürgen Janke [6].

b) Cell culture with different glucose content

Preadipocytes were incubated at 37°C in a humidified atmosphere with 5% CO2 as described by Janke [6]. Cells were stimulated to proliferate over 24 h, then glucose was added at various concentrations to the culture medium respectively (Glucose-free adipocyte medium, supplemented with 1 μmol/l insulin; 0.25% Glucose adipocyte medium, supplemented with 1 μmol/l insulin; 0.5% Glucose adipocyte medium, supplemented with 1 μmol/l insulin).

c) Gene expression analsed by real-time PCR.

Preadipocytes were trypsinized, washed once with PBS, and pelleted (380g, 10 min) after glucose was added at various concentrations to the culture medium at 2, 4, 8, 16, and 32 h. Total RNA was extracted with TRIzol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. An ABI PRISM 7000 sequence detection

system (Applied Biosystems, Foster City, CA, USA) was used to perform the real-time PCR. The reaction mixture was prepared to use a Universal PCR Master Mix (Applied Biosystems) according to the manufacturer's instructions. The mixture was incubated at 50 °C for 2 min and at 95 °C for 10 s, and then the PCR was performed by 34 cycles at 94 °C for 5 s and at 58 °C for 40 s, 72 °C for 10 s, and an additional extension period at 72 °C for 2 min. Melting curve from 65℃ to 95℃ read every 0.5℃ hold 5 s. Relative standard curves were generated in each experiment to calculate the input amounts of the unknown samples.

Expression analysis was performed for the genes encoding HSL (GenBank Accession No.HM591297). Human β-actin was chosen as the endogenous control ("housekeeping gene"). The primers were as follows: HSL gene, forward 5′- GTCACGCACAGCATGGACCT 3′, reverse 5′AAGCGGCCACTGGTGAAGAG- 3′; β-actin gene, forward 5′-TGCGGGACATCAAGGAGAAG-3′, reverse 5′-AGTTGAAGGTGGTCTCGTGG-3′.

The conventional RT-PCR was performed for standard RNA using the primers (HSL-F, HSL-R, actin-F and actin-R) mentioned above. The standard template was prepared as described by Yang [7].

Standard RNA (1×109 copies/μL) was used to establish a standard curve. The Primers (HSL-F, HSL-R, actin-F and actin-R) were used for this amplification, and a range from 1 ×108 to 1×102 gene copies/reaction was used [7].

d) Statistical analysis

Data were expressed as means ± SDs. The statistical significance of the differences was determined by Student's t-test. P values <0.01 were considered statistically significant.

III. RESULTS

a) Standard curve of the established FQ-PCR

The standard curves of HSL and β-actin FQ-PCR assays were constructed using serially diluted plasmid with tenfold dilution ranging from 10^8 to 10^2 copies as templates. The correlation coefficients of the two standard curves were 0.9998 and 0.9999, and the PCR efficiencies were both greater than 0.95 (Fig. 1). The high PCR reaction efficiencies indicated that these real-time PCR assays were suitable for genomic DNA quantification. The good linearity between DNA quantities and fluorescence values (Ct) indicated that these assays were well suitable for quantitative measurements. By using the following formula, we could quantify the mRNA expression of HSL of unknown samples: Y = -3.051X + 43.60 (Y = threshold cycle, X = log starting quantity).

b) Dynamic changes of HSL gene expression in adipocyte by using the established FQ-PCR assay

HSL gene expression quantification using the established FQ-PCR demonstrated that the HSL DNA copy number of each sample could be calculated using the cycle threshold (Ct) value determined from the standard curve. The dynamic changes of HSL gene expression within the adipocyte after that glucose were added at various concentrations to the culture medium was intermittently determined by the FQ-PCR in separate segments of adipocyte over a 32 h period. Results revealed that the copy numbers of HSL reached a peak at 2–8 h when the glucose density was 0%, 0.25%, and 0.5% with 19.87±1.25, 16.76±1.74, 15.14±1.41 (copies/μL), respectively. The glucose density of the high group significantly cut down the mRNA expression of HSL ($P < 0.01$) rather than in the others (Fig. 2).

IV. DISCUSSION

In this study, one quantitative real-time PCR system employing the human β-actin gene, as housekeeping gene, and HSL, was developed to analyze the expression of HSL. Real-time PCR is a quickly developing technology and is used in many fields. It is convenient, fast and sensitive for the detection of mRNA expression [8], indicating that the real-time PCR method is an alternative for estimation of the detection of mRNA express.

HSL regulates the hydrolysis of acylglycerol and cholesteryl ester (CE) in various organs, including adipose tissues. The aim of this study was to analyze the expression of HSL, which is a central enzyme of lipogenesis with a different glucose level. We revealed a significant correlation of HSL expression with the high degree of glucose in primary pig preadipocytes in vitro. Surprisingly, the copy numbers of HSL reached a peak at 2–8 h at various concentrations of glucose. The high concentrations of the glucose group significantly cut down the mRNA expression of HSL ($P<0.01$) rather than in the others. Our finding is just an initiation of HSL in obesity to be elucidated. Further studies are required to unravel the relationship between nutrient and hormone and other gene such as Fatty Acid Synthase 3.

V. CONCLUSION

In summary, our results provide significant data for that confirm an association between different glucose level and HSL expression in preadipocytes. However, much more work will be needed to validate such a conclusion.

ACKNOWLEDGEMENTS

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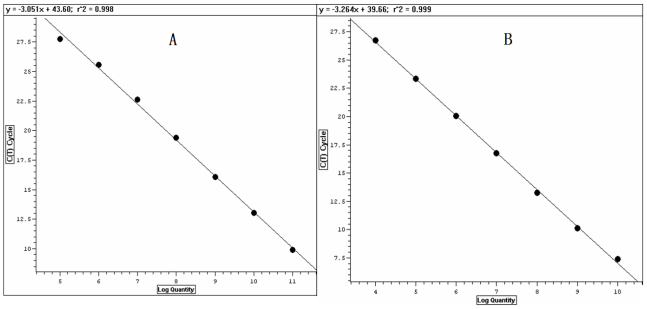
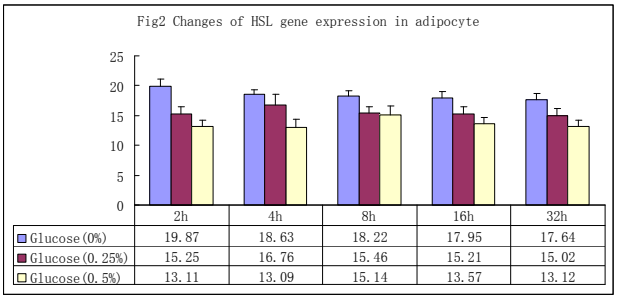


Figure 1. FQ-PCR standard curve graph: (a) standard curve of HSL by the fluorescence quantitative PCR; (b) standard curve of β-Actin by the fluorescence quantitative PCR



	2h	4h	8h	16h	32h
Glucose(0%)	19.87	18.63	18.22	17.95	17.64
Glucose(0.25%)	15.25	16.76	15.46	15.21	15.02
Glucose(0.5%)	13.11	13.09	15.14	13.57	13.12

Figure 2. Changes of HSL gene expression in adipocyte