

Original Article

Changes in potassium concentration and gene expression in mice fed a high-fat diet

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Obesity is a risk factor for various diseases, including cardiovascular disease, diabetes, renal disease, hypertension, cancer, and neural disease. Adipose tissue in animals is important for the mobilization of lipids, milk production, deposition of fat in different depots, and muscle and meat production. Understanding the genetic and physiological causes of metabolic disease is a priority in biomedical genome research. In this study, we examined several variables in mice fed a high-fat diet, including serum composition, body weight, total calorie intake, and differentially expressed genes. Body weight and blood glucose levels were not significantly different between animals fed high-fat and normal diets. However, high-fat diet groups showed reduced calorie and food intakes. Levels of sodium, ionized calcium, glucose, hematocrit, hemoglobin, pH, PCO₂, PO₂, TCO₂⁺, HCO₃⁺, base excess, and SO₂ in the blood were not significantly different between mice fed high-fat and normal diets. Serum potassium concentration, however, was lower in mice a high-fat diet. Differentially expressed genes were also compared between the two groups. The purpose of this study was to discover new genes as a result of annealing control primer (ACP) PCR using 20 random primers. Five down regulated genes were identified and three of others were up-regulated by high-fat diet. Known genes were excluded from this result. In addition, the relationships among candidate genes and high-fat diet should be investigated according to potassium concentration in the blood. In conclusion, mice fed normal and high-fat diets showed no significant difference in body weight, whereas high-fat diet led to changes

in blood composition and differential expression of several genes. These findings may provide a better understanding of the mechanisms underlying the association between obesity and metabolic diseases.

Key words: high fat, obesity, blood composition, annealing control primer-polymerase chain reaction, potassium level

Introduction

According to population-based studies, diabetes is caused by an imbalance in energy status, such as excess energy from overeating or an energy deficit. Adipose tissue is important for energy storage. Studies on animal adipose tissue have focused mainly on the mobilization of lipids, milk production, deposition of fat in different depots, and muscle and meat production [1]. In humans, obesity is an established risk factor for various diseases, including cardiovascular disease, diabetes, renal disease, hypertension, cancer, and neural disease [2-4].

Mice fed a high-fat diet reportedly show significant differences in potassium concentration [5]. The thin obesity phenotype is commonly observed in modern people's peculiar high-fat diet. In addition, altered potassium concentration contributes to the occurrence of Korean adult diseases such as hypertension, diabetes, and vascular disease.

Potassium is a primary electrolyte and is concentrated within cells. Only 2% of the body's total potassium is

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available in the serum or bloodstream, and small changes in serum potassium levels can affect body function. One of the more important functions of potassium is maintenance of electrical activity in cells. As the major intracellular cation in the body, potassium is required for normal cellular function and metabolic balance. Cells with high electrical activity are particularly affected when potassium levels decrease [6]. Low serum potassium levels and low dietary potassium intake are associated with many metabolic disorders [7]. A low serum potassium concentration is related to disorders of glucose-lipid metabolism, and low potassium values are associated with higher glucose values in people under thiazide diuretics therapy [8]. Previous studies have shown that potassium depletion can cause insulin resistance, and therapeutic interventions aimed at correcting potassium levels could improve insulin sensitivity [9, 10]. A meta-analysis collecting data from randomized controlled trials confirmed a significant inverse correlation between serum potassium level and blood glucose, whereas potassium supplementation alleviated such an effect [8]. Serum potassium levels are inversely correlated with incidence of diabetes. Individuals with lower serum potassium levels possess greater insulin resistance. Acute and chronic hypokale-

mia can limit control of diabetes by impairing insulin release and reducing insulin sensitivity [11]. Abdominal obesity as another risk factor is associated with low plasma potassium levels in patients under thiazide therapy [12]. In addition, the impact of potassium depletion on metabolic disorders is mediated partly by deterioration of endothelial function via reduction of nitric oxide, which is needed to maintain endothelial function [13].

Therefore, understanding the genetic and physiological causes of metabolic diseases as well as their regulation by environmental factors, such as dietary habits, physical activity, or the microbiome, is a priority in biomedical genome research. Genetically modified animal models and mutant mouse lines are extremely important for investigating genetic causes, pathogenesis, early diagnosis, and therapies for metabolic disorders. International programs are aimed at compiling a complete overview of the functions of mammalian genes using knockout mutations in mice for all protein-coding genes [14]. These mouse models are useful tools for identifying genetic functions related to metabolic disease.

An annealing control primer (ACP) system is a highly accurate and sensitive polymerase chain reaction (PCR) method used to amplify minute amounts of genetic ma-

Table 1. List of primers for the ACP-PCR system

	Primer	Sequences
cDNA synthesis	dT-ACP1	CTGTGAATGCTGCGACTACGATIIII(T)18
ACP-PCR	dT-ACP2 (reverse)	CTGTGAATGCTGCGACTACGATIIII(T)15
	ACP1	GTCTACCAGGCATTCGCTTCATIIIIIGCCATCGACC
	ACP2	GTCTACCAGGCATTCGCTTCATIIIIAGGCGATGCC
	ACP3	GTCTACCAGGCATTCGCTTCATIIIIICGGAGGATG
	ACP4	GTCTACCAGGCATTCGCTTCATIIIIIGCTGCTCGCG
	ACP5	GTCTACCAGGCATTCGCTTCATIIIIAGTGCGCTCG
	ACP6	GTCTACCAGGCATTCGCTTCATIIIIIGGCCACATCG
	ACP7	GTCTACCAGGCATTCGCTTCATIIIIIGGTCACGGAG
	ACP8	GTCTACCAGGCATTCGCTTCATIIIIIGATGCCGCTG
	ACP9	GTCTACCAGGCATTCGCTTCATIIIIITGGTCGTGCC
	ACP10	GTCTACCAGGCATTCGCTTCATIIIIICTGCAGGACC
	ACP11	GTCTACCAGGCATTCGCTTCATIIIIACCGTGGACG
	ACP12	GTCTACCAGGCATTCGCTTCATIIIIIGCTTACCGC
	ACP13	GTCTACCAGGCATTCGCTTCATIIIIIGCAAGTCGGC
	ACP14	GTCTACCAGGCATTCGCTTCATIIIIIGCAAGTCGGC
	ACP15	GTCTACCAGGCATTCGCTTCATIIIIICACCGTGTG
	ACP16	GTCTACCAGGCATTCGCTTCATIIIIIGTCGACGGTG
	ACP17	GTCTACCAGGCATTCGCTTCATIIIIICAAGCCACG
	ACP18	GTCTACCAGGCATTCGCTTCATIIIIICGGAGCATCC

terial available in tissue samples [15, 16]. Primers anneal specifically to a template, and only genuine products are amplified. Differentially expressed genes (DEGs) between control versus experimental groups have been identified using the ACP PCR technique.

In this study, we examined several physiological variables in high-fat diet mice, including serum composition, body weight, total calorie intake, and differentially expressed genes, to understand the impact of a high-fat diet.

Materials and Methods

Animals and study design

Mice (C57BL/6J strain) were used in this study (Sam-TaKo, Osan, Korea). Animals were reared and maintained

under specific pathogen-free conditions. All animal experiments were carried out in accordance with the guidelines for animal experimentation and with permission from the Kyungpook National University Animal Use and Care Committee. Mice were housed in laboratory cages at a temperature of 24°C and 50% humidity under a 12 hr light/dark cycle. Mice were divided into two groups, and each group ($n=10$) was given free access to a different diet for 10 weeks. Male mice were fed a normal diet (ND, 10% fat, Research Diets Inc.) and high-fat diet (HD, 60% fat, Research Diets Inc.) starting at 8 weeks of age. Body weight and food intake were monitored weekly. Muscle atrophy was induced by surgery in the hind limb at approximately the mid-femur. Unilateral collection of the hind limb was performed at 10 weeks after the end of each diet ($n=5$ per

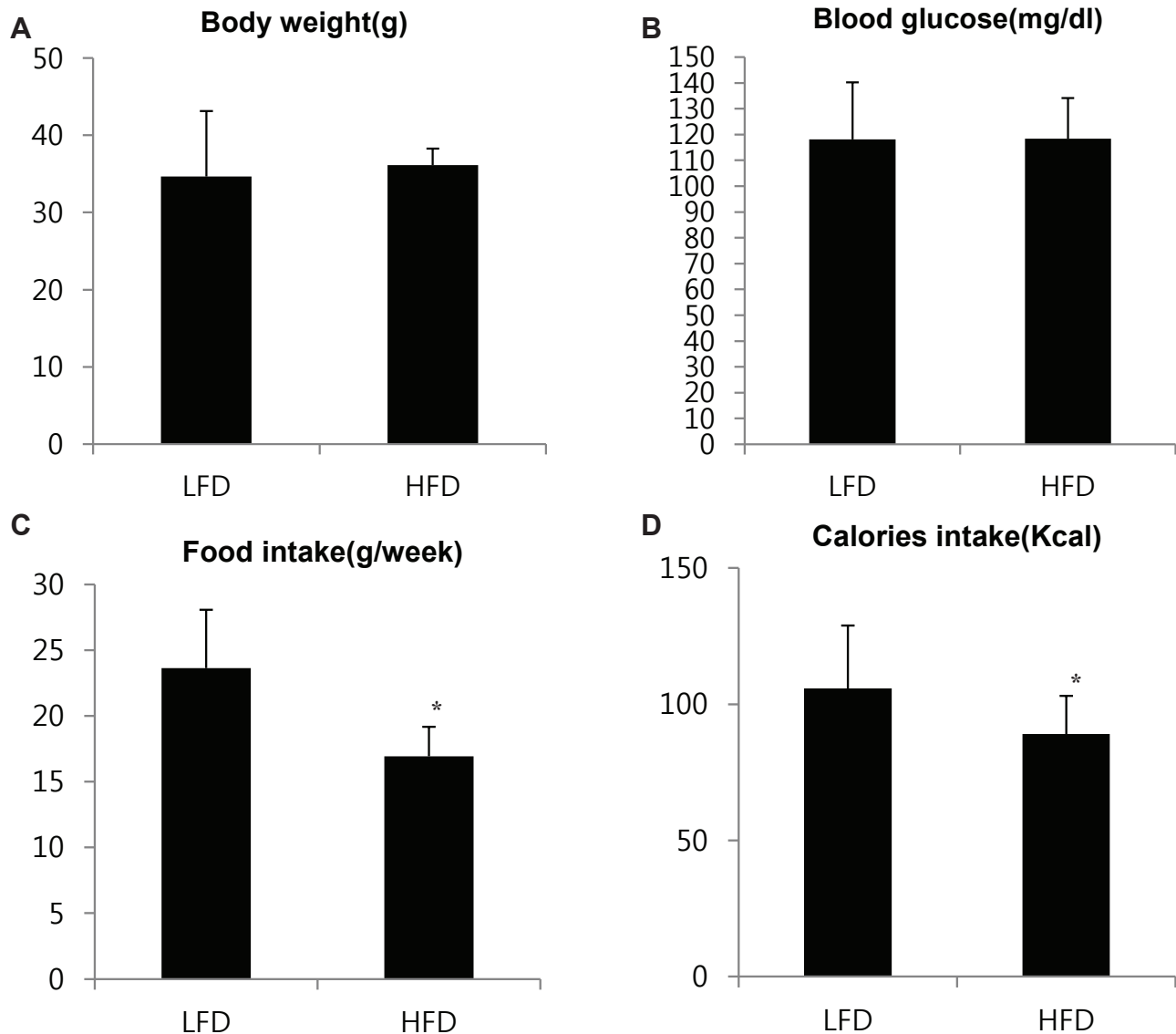


Fig. 1. Energy-related analysis of mice fed normal and high-fat diets. Body weight and blood glucose levels were not significantly different. The high-fat diet groups showed reduced calorie and food intakes. Data are shown as the means $\pm$ S.D. of values obtained from triplicate experiments. Differences were evaluated using Student's *t*-test (*, $p<0.05$)

group). This study was approved by the Institutional Animal Care and Use Committee (IACUC) of Kyungpook National University (approval no:2014-0037-1).

Tissue sampling

Muscle samples from the femoral region were taken

from both ND and HD mice. Blood samples were collected from the retro-orbital plexus, and blood analyses were carried out using an i-STAT system with a CG8+ cartridge for sodium, potassium, ionized calcium, glucose, hematocrit, hemoglobin, pH, PCO_2 , PO_2 , TCO_2^+ , HCO_3^+ , base excess, and SO_2 .

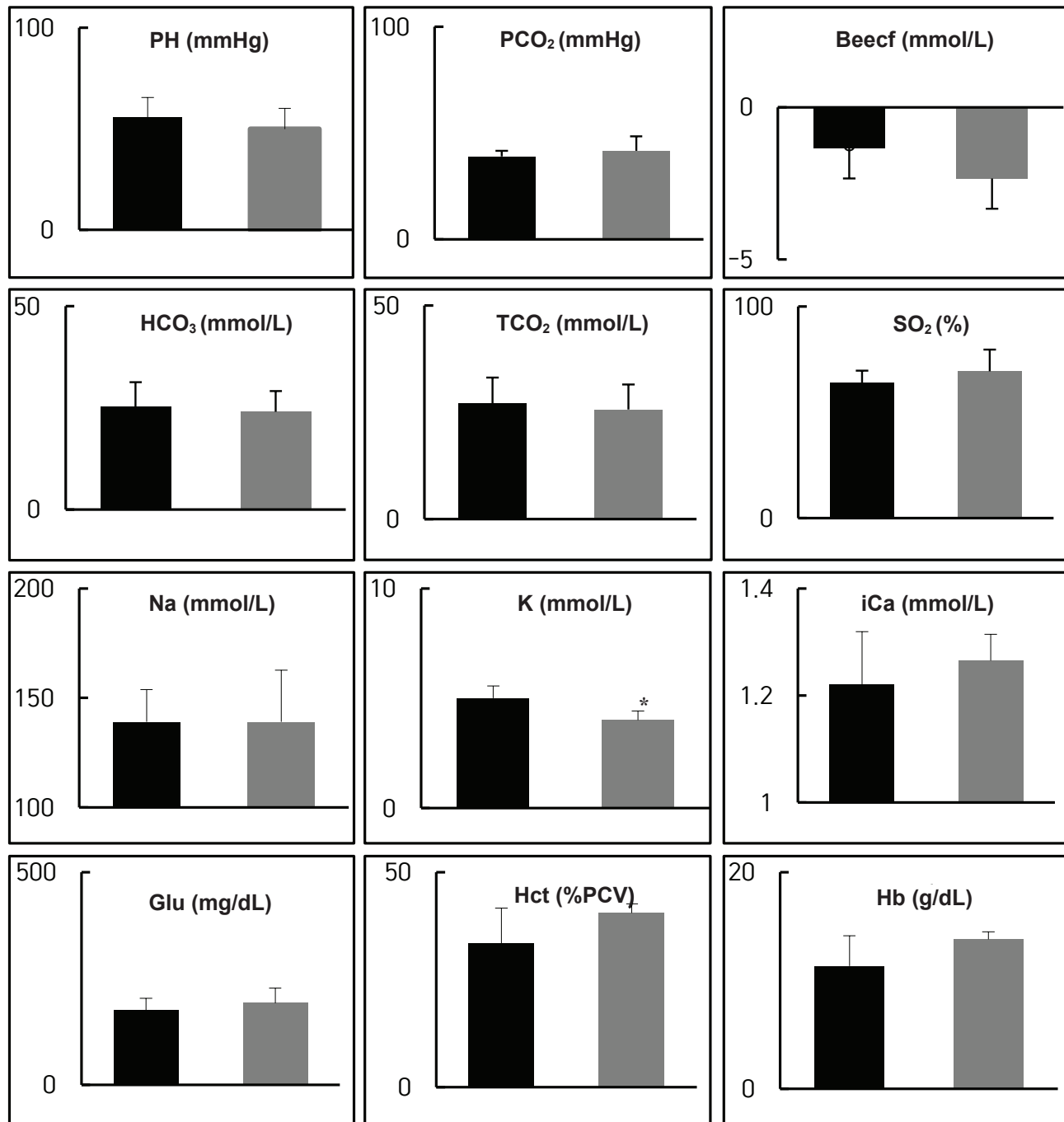


Fig. 2. Analysis of serum samples of mice fed normal and high-fat diets. Sodium (Na), Ionized Calcium (Ca), Glucose (Glu), Hematocrit (Hct), Hemoglobin (Hgb), pH, PCO_2 , PO_2 , TCO_2^+ , HCO_3^+ , base excess (BE), and SO_2 were not significantly different between mice fed high-fat and normal diets. However, differences in potassium (K) concentration were detected. Data are shown as the means $\pm$ S.D. of values obtained from triplicate experiments. Differences were evaluated using Student's t-test (*, $p < 0.05$).

Total RNA preparation

Total RNA was isolated from the femoral region with Trizol reagent (Invitrogen, Carlsbad, CA). Extracted RNA was quantified spectrophotometrically.

Reverse transcription

Reverse transcription was performed by RNA denaturation for 5 min at 65°C and elongation for 90 min at 42°C with dT-ACP1 primer. After a stop reaction for 5 min at 95°C, samples were held at 4°C until the next experiment. A final reaction volume of 20 µL consisted of 2 µg of RNA, 4 µL of 5 × reaction buffer, 5 µL of dNTP (each 2 mM), 2 µL of 10 mM cDNA synthesis primer dT-ACP1, 0.5 µL of RNase inhibitor, and 1 µL of SuperScript II Reverse Transcriptase (Invitrogen, Carlsbad, CA). The final reaction mixture was diluted in 190 µL of water.

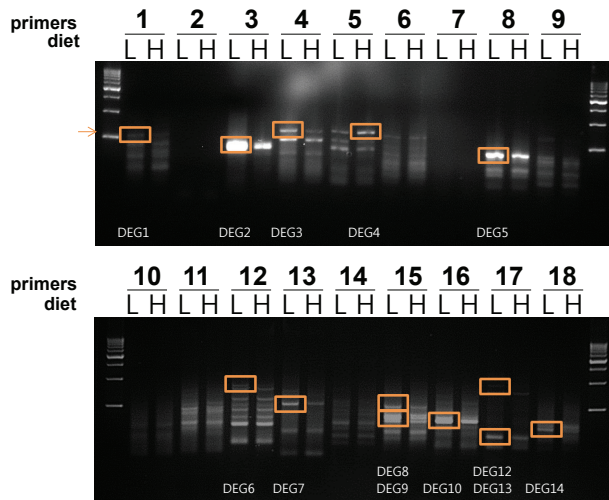


Fig. 3. Differentially expressed genes in muscle tissue from mice fed normal and high-fat diets. Differential expression of genes was determined with 14 cDNA fragments amplified by ACP-PCR using primers 1, 3, 4, 5, 8, 12, 13, 15, 16, 17, and 18. PCR products were visualized by electrophoresis and ethidium bromide staining of gels using a UV illuminator. WT (M: marker, C: control diet, H: high-fat diet)

Annealing control primer (ACP)-PCR

cDNAs were amplified to second-strand cDNA by random PCR amplification using dT-ACP2 and an ACP primer (Table 1). The second round of PCR consisted of 40 amplification cycles at 94°C for 1 min, 50°C for 3 min, and 72°C for 1 min. A third round of PCR was performed at 94°C for 40 sec, 65°C for 40 sec, and 72°C for 1 min. The PCR protocols in this study were performed as described by Na *et al.* [17]. PCR products were loaded and run on 2% agarose gels and then stained with 1 mg/mL of ethidium bromide.

Cloning and sequencing of differentially expressed genes (DEGs)

PCR products confirmed as being differentially expressed were used for cloning. PCR products were extracted and cloned into TOPO TA vector using a TA Cloning Kit (Invitrogen, Carlsbad, CA). The constructed vectors were transformed into competent TOP10 *Escherichia coli* cells. Colonies were cultured on LB agar plates with ampicillin overnight in a 37°C incubator. Colonies were selected and cultured in LB broth overnight in a shaking incubator. DNA was prepared, after which the sequences were determined. To identify the DNA inserts, sequences were analyzed using a National Center for Biotechnology Information (NCBI) Blast search.

Results

Effect of high-fat diet

For mice fed a ND or HD, body weight and blood glucose levels were not significantly different. However, the high-fat diet groups showed reduced calorie and food intakes (Fig. 1). To compare bloodstream compositions between the two groups, blood samples from the Retro-Orbital plexus were checked using the i-STAT system. Sodium, ionized calcium, glucose, hematocrit, hemoglobin, pH, PCO₂, PO₂, TCO₂⁺, HCO₃⁺, base excess, and SO₂

Table 2. List of differentially expressed genes in mice fed a high-fat diet and normal diet

DEG No.	Expression Up or Down	Sequence homology search	
		Accession No.	Gene name
DEG 2	Down	EU558532.1	<i>Arabidopsis lyrata</i> cline MITE7 transposon-insertion display band genomic sequence
DEG 2	Down	EU558531.1	<i>Arabidopsis lyrata</i> cline MITE6 transposon-insertion display band genomic sequence
DEG 3	Down	AM946177.1	<i>Gibberella miniliformis</i> p450-4 gene for ent-kaurene oxidase
DEG 3	Down	EU874251.1	<i>Burkholderia cenocepacia</i> strain K56-2 hypothetical protein gene
DEG 4	Up	AM747472.1	<i>Flagellophora apelti</i> partial 28S rRNA gene
DEG 4	Up	AB298934.1	<i>Bombyx mori</i> dh40 mRNA for diuretic hormone 40
DEG 4	Up	HE971736.1	<i>Platynereis dumerilli</i> partial mRNA for transcription factor Id
DEG 13	Down	FM242736.1	Archaeon partial 16S rRNA gene

were not significantly different between the ND and HD groups. Potassium (K) concentration, however, was different between the two treatment groups (Fig. 2).

Identification of differentially expressed genes

To identify differentially expressed genes, RNA was extracted from the femoral region of mice, and cDNAs were synthesized using 2 µg of RNA. The cDNAs were amplified by ACP-PCR using ACP primers (Table 1). Each primer set amplified different PCR products, as shown in Fig. 3. Differentially expressed bands were identified from 14 fragments that were amplified using primers 1, 3, 4, 5, 8, 12, 13, 15, 16, 17, and 18 (Fig. 3). PCR products were visualized by electrophoresis and purified using a gel purification system (Qiagen, USA). Finally, four genes were identified by DNA sequencing and Blast search (Table 2). Transposon-insertion related genes and hypothetical protein genes showed high similarities with these genes.

Discussion

Diabetes is classified into two categories, type-1 diabetes and type-2 diabetes, characterized by insulin secretion and insulin resistance, respectively. The disease mechanisms, pathogenesis, and treatment of diabetes have been reviewed in detail elsewhere [18-20].

Commonly, different strains of mice demonstrate different responsiveness to high-fat diets [21]. Here, we used C57BL/6N mice to study the effects of high-fat diet consumption. Around 10% of C57BL/6N mice showed no increase in body weight, despite being on a high fat diet. In general, the body weight of mice fed a high-fat diet was higher than that of mice fed a general diet. This result can be attributed mostly to differences in feed efficiency or expression of diet-related specific genes. However, this study focused on the fact that mice that were resistant to high-fat diet showed differences with normal mice in the phenotype of entity which the bodyweight was not increased.

This study demonstrated differences between mice fed a normal diet or high-fat diet without changes in body weight. However, there were several differences in blood composition and gene expression.

Mice fed a high-fat diet showed lower serum potassium levels (Fig. 2). High-fat diet mice showed no difference in total calorie intake or body weight compared to the mice fed a normal diet (Fig. 1). Adults with lower serum potassium levels and potassium intake are at higher risk for diabetes onset [22]. Low potassium level has been associated with increased insulin resistance in both basic research [23] and epidemiological studies [24, 25]. Acute and chronic hypokalemia can worsen diabetic control partly by reducing tissue sensitivity to insulin [11]. Ad-

ditionally, central obesity patients have lower potassium levels in their plasma [12]. There is an evidence that potassium depletion can lead to low nitric oxide levels and endothelial dysfunction [26, 27]. These negative effects may lead to insulin resistance, hypertriglyceridemia, and glucose intolerance. Moreover, chronic hypokalemia in primary aldosteronism (PA) patients can indirectly reduce adiponectin levels, which may result in insulin resistance. Therefore, elevation of both potassium and adiponectin levels can improve insulin sensitivity. The relationship between potassium and glucose levels and lipid metabolism has been described [7]. Lower potassium levels can increase the risk of metabolic syndrome [7]. According to these results, lower potassium levels are related to HD. Additional studies are required to clarify this.

The ACP-PCR technique was developed by the Seegene Company as an accurate and widely applicable technique. In this study, primers 3 and 8 with ACP2-amplified specific bands decreased in HD mice samples. These genes were clarified by DEG cloning in Topo vector and by sequencing methods (Table 2). The KCN gene family has been shown to be differentially expressed based on the potassium concentration in the blood [28]. The purpose of this study was to discover new genes as a result of ACP PCR using 20 random primers. Known genes were excluded from this result. In addition, the relationships among candidate genes and high-fat diet according to potassium concentration in the blood will be clarified in the future.

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