Body Fat-Reducing Effects of Whey Protein Diet in Male Mice

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Body Fat-Reducing Effects of Whey Protein Diet in Male Mice

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Abstract: This study investigated the mechanism of reducing body fat via whey protein diet. Pregnant mice were fed whey or casein, and their offspring were fed by birth mothers. After weaning at 4 weeks, male pups received the diets administered to their birth mothers (n = 6 per group). At 12 weeks of age, body weight, fat mass, fasting blood glucose (FBG), insulin (IRI), homeostatic model assessment of insulin resistance (HOMA-IR), cholesterol (Cho), triglyceride (TG), the expression levels of lipid metabolism-related genes in liver tissues and metabolomic data of fat tissues were measured and compared between the groups. The birth weights of pups born were similar in the two groups. Compared to the pups in the casein group, at 12 weeks of age, pups in the whey group weighed less, had significantly lower fat mass, HOMA-IR and TG levels ($p < 0.01$, $p = 0.02$, $p = 0.01$, respectively), and significantly higher levels of the antioxidant glutathione and the anti-inflammatory 1-methylnicotinamide in fat tissues ($p < 0.01$, $p = 0.04$, respectively). No differences were observed in FBG, IRI, Cho levels ($p = 0.75$, $p = 0.07$, $p = 0.63$, respectively) and expression levels of lipid metabolism-related genes. Whey protein has more antioxidant and anti-inflammatory properties than casein protein, which may be its mechanism for reducing body fat.

Keywords: antioxidant effect; anti-inflammatory effect; glutathione; 1-methylnicotinamide; metabolite analyses

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1. Introduction

Japan is one of the developed countries where average birth weight has decreased and the birth rate of low-birth-weight (LBW) infants has not declined [1]. LBW infants have an elevated risk of developing diseases such as obesity and type 2 diabetes mellitus in adulthood. The fetus undergoes physiological changes to adapt to its environment when undernourished in utero, including slowed weight gain, resulting in relative overnutrition when the nutritional environment improves after birth. The developmental origins of health and disease (DOHaD) theory [2] affirm that disease risks need to be fully understood to avoid their development over the lifespan. For pediatricians, the DOHaD theory supports the idea that nutritional management in early childhood and pre-adolescence is necessary to prevent disease development in the first place [3]. This study focused on whey protein, a nutrient that can potentially protect LBW infants against metabolic syndrome later in life.

Whey protein, a nutrient-rich dairy protein that is abundant in dairy products such as yogurt and cheese, is associated with many health benefits. Whey protein is considered a functional food and has been increasingly demanded as a dietary supplement in recent years [4]. Whey protein is also present in breast milk and artificial formulas. Protein composition in breast milk changes over the lactation period. Colostrum consists of 90% whey protein and 10% casein protein; however, the ratio shifts to 60% whey and 40%

casein in mature breast milk. In contrast, cow milk usually consists of 20% whey and 80% casein proteins [4]. Major components of whey protein include lactoferrin, beta-lactoglobulin, alpha-lactalbumin, glycomacropeptide, and immunoglobulin [5]. Increasing evidence demonstrates the health-promoting effects of whey protein at the biochemical level, including:

1 Glucose metabolism effect Whey protein improvement of insulin resistance by inhibiting the secretion of serotonin in peripheral tissues and fibroblast growth factor 21 in liver tissue [6].
2 Muscle protein synthesis effect Whey protein promotion of muscle synthesis by activating the mammalian target of rapamycin (mTOR), a metabolic pathway required for muscle synthesis [7].
3 Anti-inflammatory effect In a murine hepatitis model, whey protein suppression of the production of inflammatory cytokines, thereby inhibiting hepatocyte necrosis and apoptosis [8]. Similar results were found in clinics, where it suppressed the inflammatory response in COPD patients [9].
4 Antioxidant effect Whey protein exhibition of antioxidant activities in vitro [10].
5 Lipid metabolism effect Whey protein promotes triglyceride degradation and inhibits fatty acid synthesis in mice by affecting transcription factors involved in lipid metabolism [11].

Recently, clinical reports found LBW infants with non-obese type 2 diabetes mellitus. Kuwabara et al. reported that LBW infants raised with adequate nutrition after birth often develop type 2 diabetes in adulthood and that, at the time of onset, they had a significant accumulation of visceral fat compared to subcutaneous fat [12]. Nagano et al. determined that non-obese LBW infants in pre-adolescence often develop type 2 diabetes in adulthood, and their body fat is in the normal range, while their muscle mass is deficient [13], suggesting that muscle mass and lipid metabolism may be involved in the pathogenesis of type 2 diabetes mellitus in individuals born as LBW infants. As a cause of this, it has been reported that preterm infants have higher levels of oxidative stress markers compared to full-term infants [14]. Moreover, males are reported to have higher levels of oxidative stress markers than females; as a result, males are more prone to type 2 diabetes and cardiovascular events [15,16]. Due to the effects of whey protein in promoting muscle synthesis and improving glucose and lipid metabolisms, along with its anti-inflammatory and antioxidant activities, feeding LBW infants a diet rich in whey protein during infancy, early childhood, and pre-adolescence may help to prevent them from developing diabetes later in life. However, the mechanism by which whey protein exerts these effects remains to be perfectly elucidated [17].

Therefore, this study aimed to investigate the effect of whey protein on glucose and lipid metabolism and identify the potential mechanism involved in body fat reduction by measuring physical and biochemical changes in male mice exposed to whey protein from embryonic development to adulthood in comparison to mice raised exposed to casein protein diet over the same period.

2. Materials and Methods

2.1. Experimental Animals

All experimental protocols and procedures were approved by the Animal Experimentation Committee of Nihon University Itabashi Hospital (approval ID: AP20MED018-1, approval date: 5 June 2020). Pregnant Institute of Cancer Research (ICR) dams at gestational day 2 (GD2) were purchased from Sankyo Labo Service Corporation, Inc. (Tokyo, Japan).

2.2. Rearing Conditions

ICR pregnant mice were divided into two groups upon arrival, the casein and whey dietary groups. After birth, male pups were selected and raised on the same diet as their mothers. All mice were reared under the temperature of 22 ± 2 °C, humidity of 55 ± 5%, and 12/12 h light/dark cycles. In the casein group, mice were reared on AIN-93G,

a standard rodent feed administered during pregnancy and growth periods in murine experiments (casein 20%, L-cystine 0.3%, corn starch 39.7486%, alpha-corn starch 13.2%, sucrose 10.0%, soybean oil 7.0%, cellulose powder 5.0%, mineral 3.5%, vitamin 1.0%, choline bitartrate 0.25%, tertiary butyl hydroquinone 0.0014%: energy 359 kcal) (Oriental Yeast Co., Ltd. Tokyo, Japan) [18]. In the whey group, the mice were reared on a modified blend of AIN-93G in which the casein component was replaced by whey. Pups were reared to 12 weeks of age before physical and biochemical measurements (Figure 1).

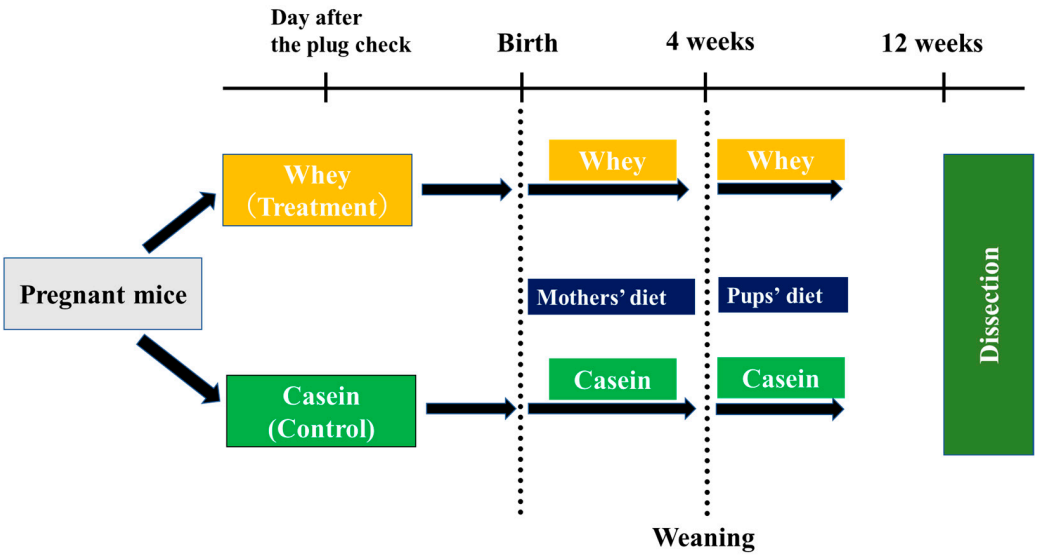

Figure 1. Experimental procedures. Study flow.

2.3. Body Weight

Pups were weighed once a week from birth to 12 weeks of age.

2.4. Blood Glucose, Serum Insulin, and Insulin Resistance (HOMA-IR)

At 12 weeks of age, adult male mice were fasted for 12 h and then dissected under isoflurane inhalation anesthesia (5% induction, 2% maintenance). Blood was collected from the heart by cardiac puncture via a midline incision. Blood glucose levels were measured using a Stat Strip XP2 (Nipro, Osaka, Japan). Next, serum was separated from total blood by centrifugation at 3000 rpm for 5 min and stored at −20 °C. Serum insulin was assessed for immunoreactive insulin levels (IRI) using a mouse/rat total insulin (high sensitivity) assay kit (Immuno-Biological Laboratories Co., Fujioka, Gunma, Japan). Serum was also assayed for insulin resistance using the human formula for the homeostasis model assessment of insulin resistance (HOMA-IR) [19].

2.5. Body Composition and Fat Weight

Body composition was measured using a bioelectrical impedance spectroscopy (BIS) device for laboratory animals (ImpediVET^{TM}: Bioresearch Center, Co., Ltd., Nagoya, Japan) [20]. To estimate fat mass (FM) and fat-free mass (FFM), we measured the BIS differences in the electrical conductivity of biological tissues since adipose tissue is less conductive than muscle and other tissues due to lower water per unit volume. Fat weight was evaluated, and all observable adipose tissue was dissected.

2.6. Serum Lipoprotein Fractionation

Serum lipoproteins were separated into distinct fractions based on their cholesterol and triglyceride contents using gel-permeation high-performance liquid chromatography (HPLC) according to a method previously described (LipoSEARCH®; Skylight Biotech, Akita, Japan) [21–23]. Cholesterol and triglyceride values were estimated in total and for each of the major lipoprotein classes: very-low-density lipoprotein (VLDL), low-density

lipoprotein (LDL), and high-density lipoprotein (HDL) based on the peaks in the HPLC elution profile corresponding to different lipoprotein particle sizes [22].

2.7. Gene Expression Analysis of Liver Tissue

RNA expression levels of the genes related to lipid metabolism in the liver (*PPARα*, *PPARγ*, *SREBP1c*, *HSL*, and *LPL*) were measured using real-time quantitative polymerase chain reaction (RT-qPCR). RNA was isolated from frozen liver tissue of male mice (n = 5 per group) using the protocol provided by ReliaPrep RNA Miniprep Systems (Promega Corporation, Madison, WI, USA). RNA was reverse-transcribed to complementary DNA using ReverTra Ace qPCR RT Master Mix (Toyobo Co., Ltd., Osaka, Japan) on an ABI Geneamp 9700 PCR-Thermal Cycler (Applied Biosystems, Thermo Fisher Scientific Inc., Tokyo, Japan). RT-qPCR was performed using KOD-Plus-Ver.2 polymerase mix (Toyobo Co., Ltd.) on an ABI Applied Biosystems 7300 Real-Time PCR System (Applied Biosystems, Thermo Fisher Scientific Inc.). In this study, we used the same primers as in a previous report [11] as a reference. These primers were manufactured by Takara Bio Inc. (Kusatsu, Japan).

2.8. Metabolomic Analysis of Adipose Tissue

A sample of frozen adipose tissue from male mice (approximately 50 mg, n = 5 per group) was placed in a homogenization tube with zirconia beads (5-mmφ and 3-mmφ), to which 1500 µL of 50% acetonitrile/Milli-Q water containing internal standards (H3304-1002, Human Metabolome Technologies, Inc. Yamagata, Japan) was added. Two cycles of homogenization at 1500 rpm for 120 s at 4 °C were performed using a beaded shaker (Shake Master NEO, BioMedical Science, Tokyo, Japan). Next, the sample was centrifuged at 2300× *g* for 5 min at 4 °C to remove high-molecular-weight components. Then, 400 µL supernatant was collected, centrifuged at 9100× *g* for 120 min at 4 °C, and filtered using a Millipore 5-kDa cut-off filter (Human Metabolome Technologies, Inc. (HMT), Tsuruoka, Yamagata, Japan). Finally, the filtrate was dried by vacuum evaporation and dissolved in 50 µL Milli-Q water. This solution was subjected to metabolomic analysis using capillary electrophoresis time-of-flight mass spectrometry [24,25] on an Agilent CE system (Agilent Technologies, Inc., Santa Clara, CA, USA). Peak area, m/z, and migration time data of the mass spectrum peaks (range: 50–1000 m/z) were calculated for peaks automatically detected using integrated software (Keio University, Shizuoka, Japan) [26]. The chemical species associated with each peak was identified based on its m/z value and migration time with reference to the HMT metabolite database. Relative levels of each metabolite were calculated by normalizing the peak area with the internal standards and sample volume.

Principal component analysis and hierarchical cluster analysis were performed according to previously described methods [27].

2.9. Serum and Urine Creatinine

Serum samples were collected as described in Section 2.4, and serum creatinine was measured using enzymatic method. Urine creatinine was measured in 24 h urine samples, collected while mice were kept in a metabolic cage for laboratory animals, using a conventional creatinine deaminase-based enzymatic method.

2.10. Statistical Analysis

Data are reported as mean ± standard error of the mean. Each outcome was compared between the experimental (whey) and control (casein) groups using Mann–Whitney U test, using JMP statistical software (ver. 14.0: SAS Institute, Cary, NC, USA). When $p < 0.05$, the differences were considered statistically significant, and when $0.05 < p < 0.10$, the differences were considered marginally significant.

3. Results

3.1. Body Weight History

Body weight at birth was not significantly different between the two groups. However, every week thereafter, the weight was lower in the whey group than in the casein group. At 12 weeks, body weight was significantly lower in the whey group than in the casein group (48.3 g vs. 61.0 g, $p < 0.01$) (Figure 2a,b).

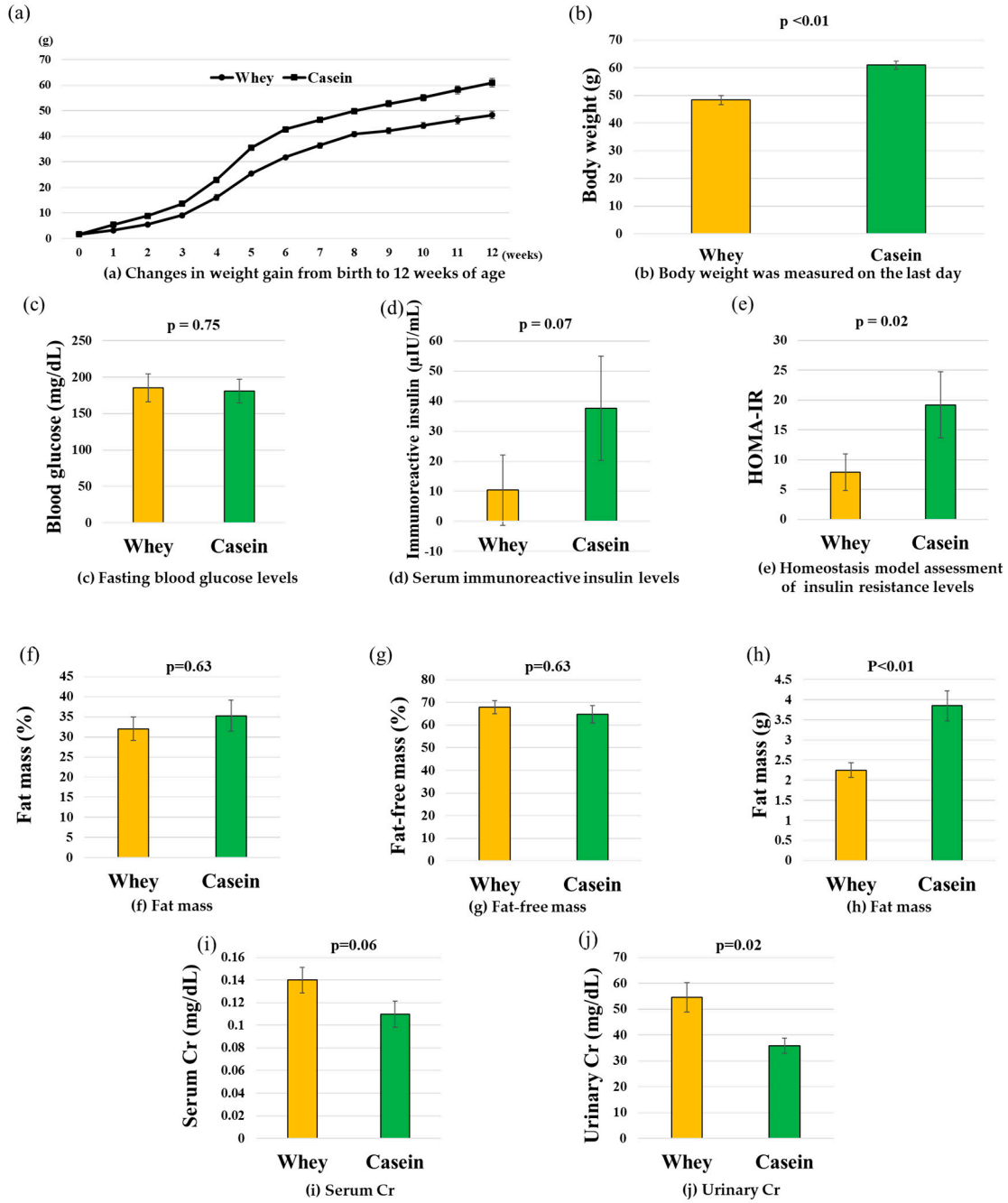

Figure 2. Body weight and glucose metabolism markers. (**a**) Changes in weight gain from birth to 12 weeks of age (●: Whey, ■: Casein). (**b**) Body weight was measured on the last day. (**c**) Fasting blood glucose levels. (**d**) Serum immunoreactive insulin levels. (**e**) Homeostasis model assessment of insulin resistance levels. (**f**) Fat mass (%). (**g**) Fat-free mass (%). (**h**) Fat mass (g). (**i**) Serum Cr. (**j**) Urinary Cr. Data are shown as the mean ± standard error of the mean (n = 6 per group).

3.2. Blood Glucose, IRI, and HOMA-IR

Fasting blood glucose levels were not significantly different between the two groups (177.5 mg/dL vs. 184.7 mg/dL, $p = 0.75$). IRI was marginally lower in the whey than in the casein group (22.0 µIU/mL vs. 47.0 µIU/mL, $p = 0.07$). HOMA-IR was significantly lower in the whey than in the casein group (7.9 vs. 19.2, $p = 0.02$) (Figure 2c–e).

3.3. Fat Weight and Body Composition

Fat weight was significantly lower in the whey than in the casein group (2.4 g vs. 3.8 g, $p < 0.01$). However, body composition was similar in both groups in terms of FFM (67.9% vs. 64.7%, $p = 0.63$) and FM (32.0% vs. 35.3 %, $p = 0.63$) (Figure 2f–h).

3.4. Serum and Urine Creatinine

Creatinine levels were marginally higher in serum in the whey group than in the casein group (0.11 mg/dL vs. 0.14 mg/dL, $p = 0.06$) and significantly higher in urine (35.8 mg/dL vs. 54.6 mg/dL, $p = 0.02$) (Figure 2i,j).

3.5. Serum Lipoprotein Fractions

For cholesterol levels, no significant differences in total or individual values were observed between the two groups (total: 173.51 mg/dL vs. 153.46 mg/dL, $p = 0.63$; VLDL: 10.85 mg/dL vs. 10.94 mg/dL, $p = 0.94$; LDL: 25.16 mg/dL vs. 23.38 mg/dL, $p = 0.52$; HDL: 136.44 mg/dL vs. 116.16 mg/dL, $p = 0.26$) (Figure 3a–d). In contrast, triglyceride levels were significantly lower in the whey group than in the casein group for every outcome measured (total: 51.47 mg/dL vs. 119.2 mg/dL, $p = 0.01$) (Figure 3e).

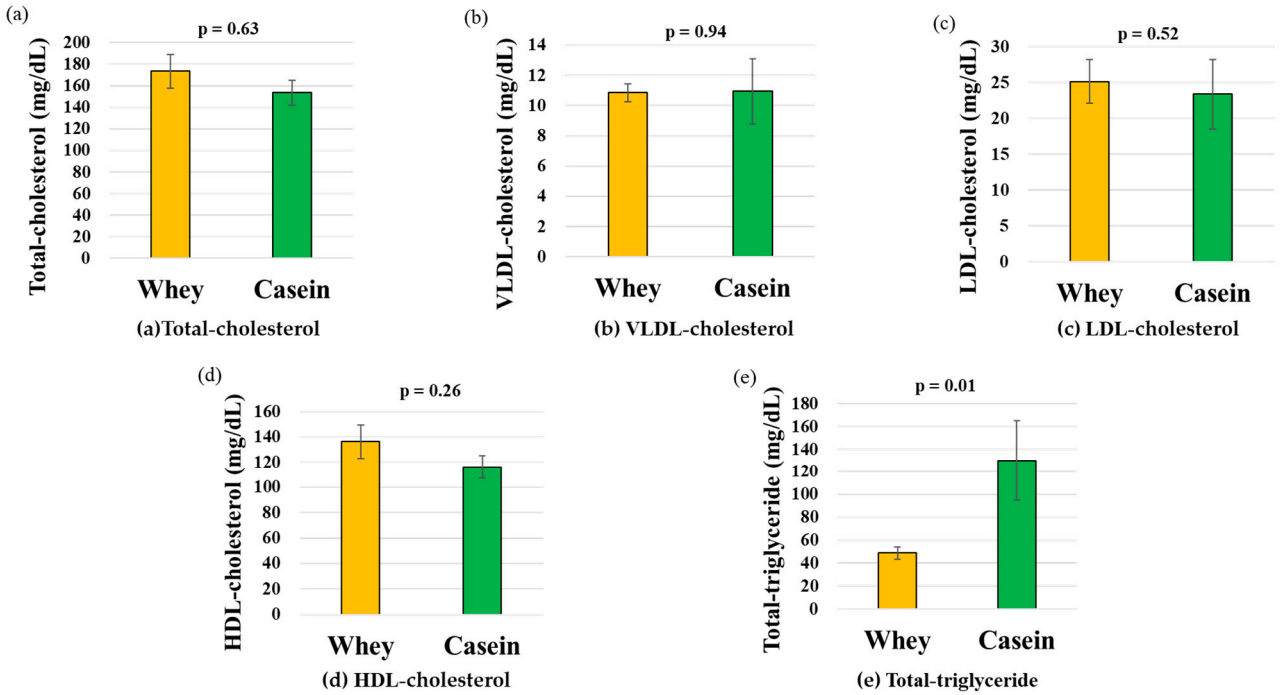

Figure 3. Body composition and serum lipoprotein levels. (**a**) Total cholesterol. (**b**) VLDL, (**c**) LDL, and (**d**) HDL-cholesterol. (**e**) Total triglyceride. Data are shown as the mean ± standard error of the mean (n = 6 per group). HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein.

3.6. Hepatic Gene Expression

RT-qPCR analysis showed that the hepatic expression of *PPARα* was marginally higher in the whey than in the casein group ($p = 0.08$); however, no other differences were observed for any of the other lipid metabolism-related genes evaluated (*PPARγ*, $p = 0.27$; *SREBP1c*, $p = 0.73$; *HSL*, $p = 0.58$; *LPL*: $p = 0.25$) (Figure 4a–e).

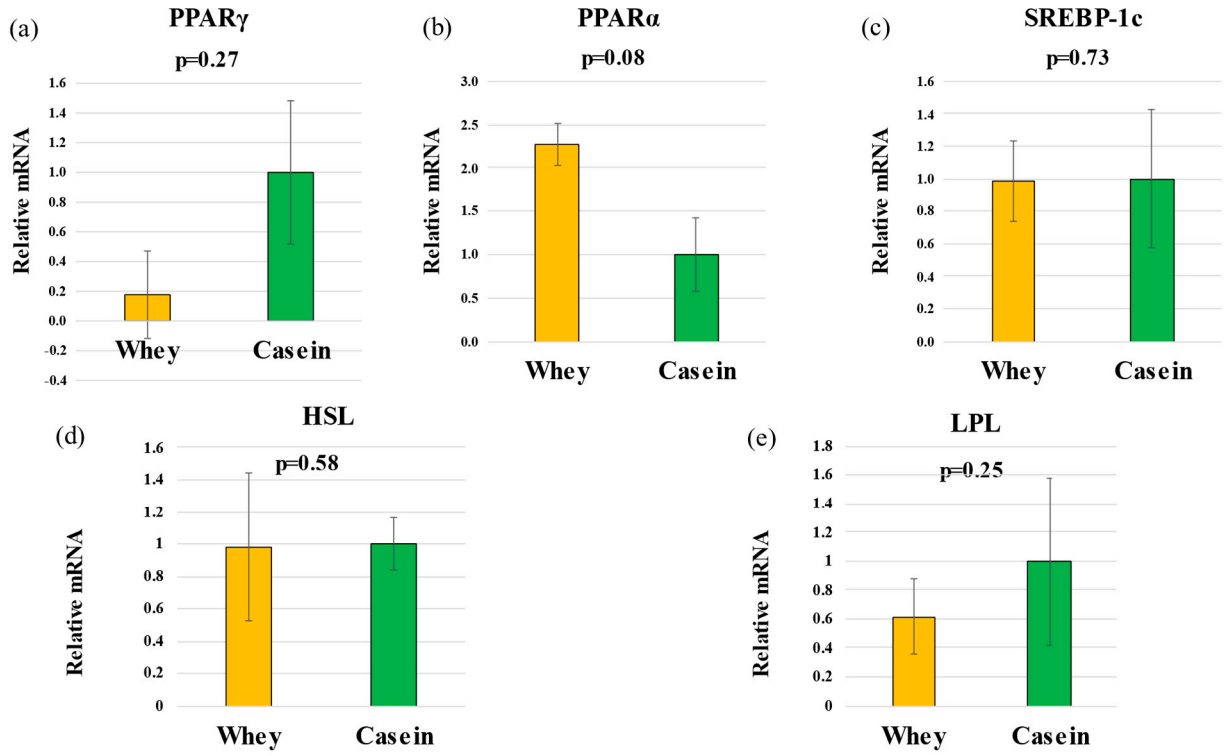

Figure 4. Relative mRNA levels. (**a**) PPARγ, (**b**) PPARα, (**c**) SREBP-1c, (**d**) HLS, and (**e**) LPL. (n = 5 per group). PPARγ, peroxisome proliferator-activated receptor γ; PPARα, peroxisome proliferator-activated receptor α; SREBP-1c, sterol regulatory element-binding protein-1c; HSL, hormone-sensitive lipase; LPL, lipoprotein lipase.

3.7. Adipose Metabolism

Results of the main component analysis or the hierarchical clustering heatmap did not show clear differences between the groups (Figure 5a,b; Supplementary Tables S1–S3). Table 1 shows the metabolites that were measured and associated with antioxidant and anti-inflammatory effects. The levels of glutathione, 1-methylnicotinamide, and myo-inositol phosphates (1-phosphate + 3-phosphate) were significantly higher in the whey group than in the casein group ($p < 0.01$, $p = 0.04$, and $p = 0.01$) (Figure 6a–c).

Table 1. Summary of metabolite analysis in the adipose tissue.

(a) Antioxidant Markers			
		Comparative Analysis Group Whey vs. Casein	
Category	Compound name	Ratio	*p*-value
Antioxidant	Ascorbic acid	1.1	0.775
	Carnosine	22	0.323
	Glutathione	7.1	0.004
	Hypotaurine	29	0.286
	Tartaric acid	0.6	0.458

Table 1. *Cont.*

(b) Anti-inflammatory markers			
		Comparative Analysis Group Whey vs. Casein	
Category	Compound name	Ratio	*p*-value
Anti-inflammatory	1-Methylnicotinamide	2.0	0.044
	Histidine	1.6	0.243
(c) Glucose metabolism markers			
		Comparative Analysis Group Whey vs. Casein	
Category	Compound name	Ratio	*p*-value
Glucose metabolism	myo-Inositol phosphates	3.1	0.013

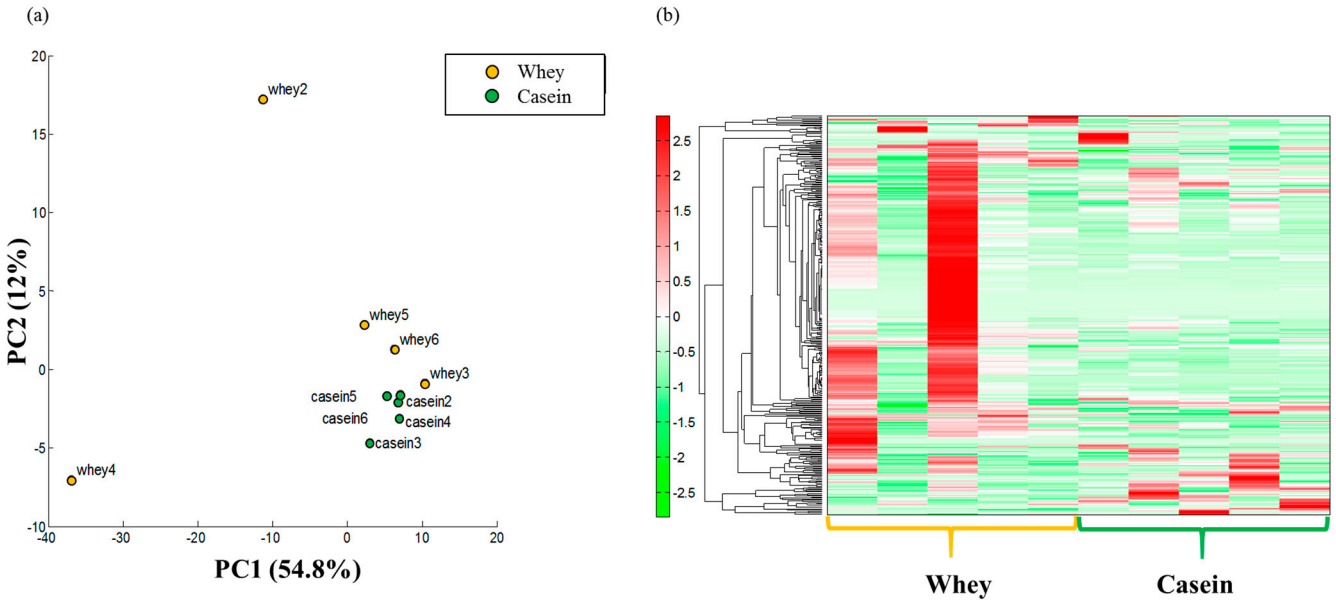

Figure 5. Metabolite analyses in fatty tissue. (**a**) Principal component (PC) analysis. (**b**) Heat map display of the hierarchical cluster analysis (n = 5 per group).

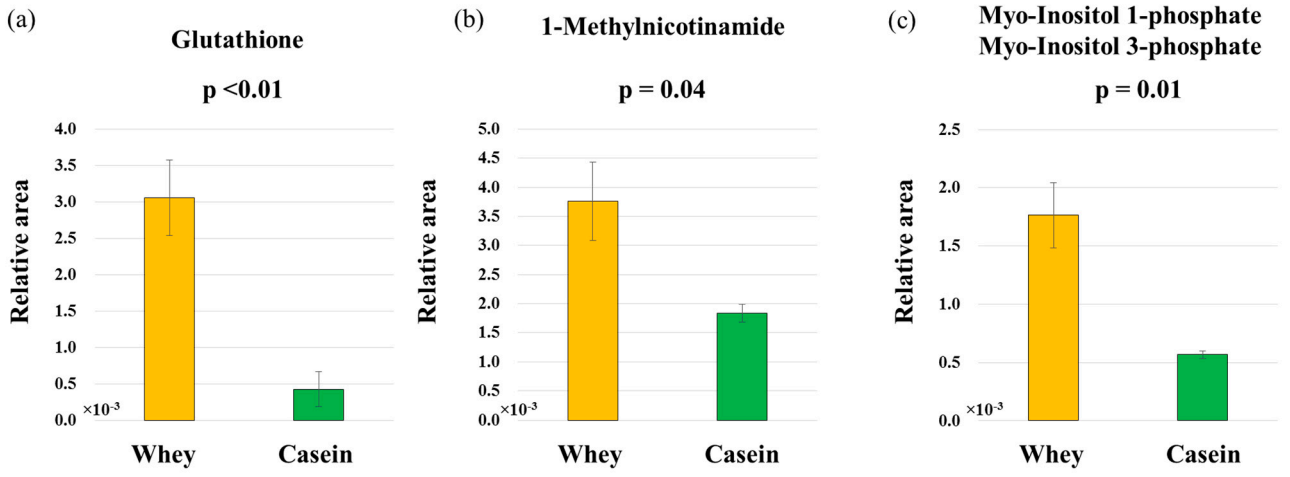

Figure 6. Comprehensive comparative analysis between the whey and casein groups. (**a**) Glutathione. (**b**) 1-Methylnicotinamide. (**c**) Myo-Inositol 1-phosphate and Myo-Inositol 3-phosphate (n = 5 per group).

4. Discussion

In the present study, whey protein intake activated lipid metabolism, reduced fat mass, and decreased insulin resistance in the mouse model. We theorize that these results were obtained because whey protein intake accelerated β-oxidation and anti-inflammatory and antioxidant activities (Figure 7a,b).

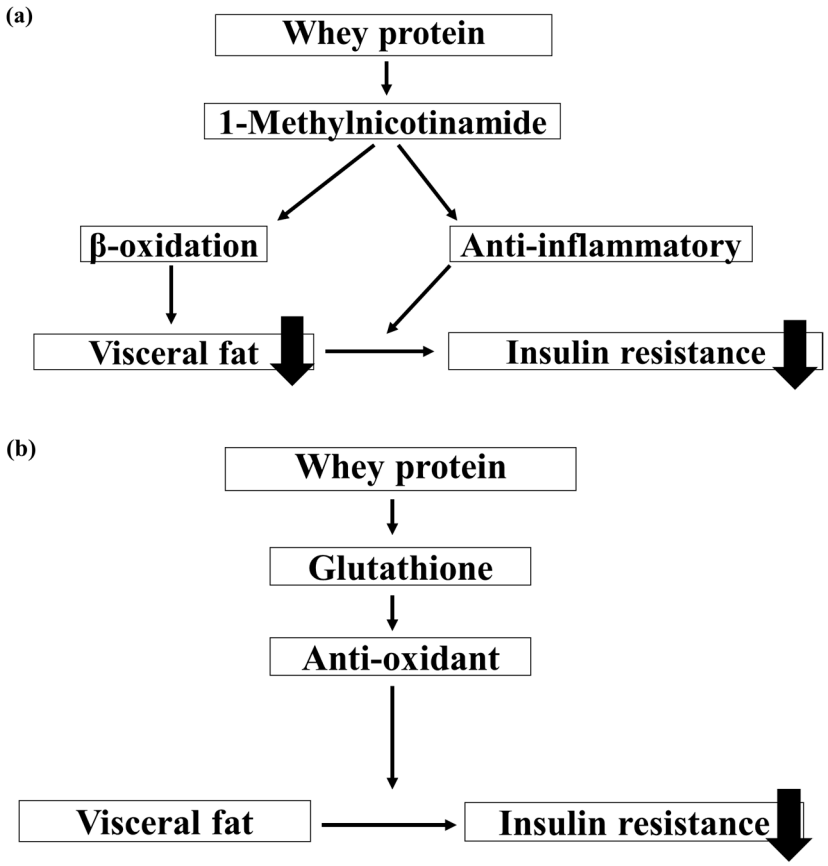

Figure 7. Schematical representation of the theory for the mechanism by which whey protein ameliorates lipid and glucose metabolisms. (**a**) 1-Methylnicotinamide and (**b**) Glutathione.

4.1. Lipid Metabolism

We found that mice raised on a whey protein diet had significantly lower level serum total-triglyceride than the ones raised on a casein protein diet. In addition, mice in the whey protein group had significantly higher *PPARα* RNA expression that those in the casein group. A previous report showed that whey protein increases *PPARα* expression [11], and that *PPARα* increases intracellular mitochondrial β-oxidation and activates lipid metabolism [28]. Therefore, we speculate that whey protein intake promotes triglyceride utilization by increasing *PPARα* expression. Furthermore, the adipose tissue of these mice in the whey protein group contained significantly higher levels of 1-methylnicotinamide, a metabolite of nicotinamide, compared to the casein group. Since 1-methylnicotinamide has anti-inflammatory and β-oxidation-limiting effects [29], we hypothesize that whey protein intake improves lipid metabolism by regulating β-oxidation (Figure 7a).

4.2. Glucose Metabolism

In adipocytes, disrupting the mechanisms regulating adipocytokine production results in the excessive production of inflammatory cytokines, leading to insulin resistance [30]. In addition, severe oxidative stress decreases and inactivates insulin receptors of adipocytes, resulting in reduced gene expression and secretion of insulin in these cells [31,32]. Our metabolomic analysis indicated that levels of 1-methylnicotinamide and glutathione in

adipose tissue were significantly higher in the whey group than in the casein group. We speculate that increased 1-methylnicotinamide due to whey protein intake suppressed chronic inflammation in adipocytes, thereby improving insulin resistance (Figure 7a). Whey protein also exerts antioxidant effects by increasing glutathione levels [33,34]; therefore, this is another plausible mechanism for the amelioration of insulin resistance observed (Figure 7b).

4.3. Improvement Myogenic Insulin Resistance

Compared to soy protein, whey protein has been reported to decrease the circulating levels of interleukin-6 and tumor necrosis factor-α and affect muscle metabolism [35]. In our study, whey protein was found to increase the level of the anti-inflammatory marker 1-methylnicotinamide in adipose tissue compared to casein protein. Greater muscle mass due to elevated serum creatine can reduce myogenic insulin resistance [36]. In this study, we did not measure muscle mass, and the two groups analyzed had statistically similar body compositions. From these results, whey protein may have the potential to reduce visceral fat more than subcutaneous fat. One possible reason why no difference was observed in FFM could be due to the relatively short duration of the intervention period for both whey and casein protein diets. However, we determined that serum creatine was significantly higher in the whey group than in the casein group. Whey protein is commonly used as a dietary supplement to increase muscle mass [37] along with increasing creatine; thus, whey protein may have influenced creatinine in this study. Elevated levels of serum creatine caused by whey protein intake may improve insulin resistance.

4.4. Myo-Inositol Phosphates

In the present study, metabolome analysis showed that myo-inositol 1-phosphate and myo-inositol 3-phosphate levels in adipose tissue were significantly higher in the whey group than in the casein group. Myo-inositol is a component of membrane phospholipids that plays a role in signal transduction. Rats with compromised myo-inositol expression show high liver triglyceride content [38]. Both myo-inositol 1-phosphate and myo-inositol 3-phosphate belong to the myo-inositol metabolic pathway. Myo-inositol improves insulin resistance [39]. Although no reports of improved glucose or lipid metabolism directly caused by myo-inositol 1-phosphate or myo-inositol 3-phosphate are available, our findings suggest a potential connection.

4.5. Infant and Oxidative Stress

Matsubasa et al. collected urine samples from fifty Japanese very-low-birthweight infants on various days after birth and measured the oxidative stress marker, 8-hydroxydeoxyguanosine. Their results showed that urine 8-hydroxydeoxyguanosines in very-low-birthweight infants were higher than those in full-term infants, and that oxidative stress marker levels decreased as the weight increased after birth [40]. Piyush et al. also reported that small-for-gestational age infants born to malnourished mothers had higher levels of the oxidative stress marker, malondialdehyde, and lower levels of enzymes in the antioxidative systems, such as superoxide dismutase, catalase, and glutathione peroxidase than appropriate-for-gestational age infants to healthy mothers [41]. These results suggest that infants with low birth weight and high prenatal stress had higher oxidative stress and lower antioxidant capacity. In our current study, it was found that nutrition with whey protein from the neonatal period improved antioxidant and anti-inflammatory capacity. Therefore, it may be possible to feed these children with whey protein to reduce oxidative stress and improve antioxidant capacity.

4.6. Comparison of the Antioxidant and Anti-Inflammatory Effects of Breast Milk and Formula

Breast milk is considered the best source of nutrition for infants in many respects. Breast milk contains carbohydrates, proteins, fats, vitamins, minerals, digestive enzymes and hormones. The protein composition of breast milk adapts to the growth of the

child, which changes over time, and the proportion of whey protein and casein protein changes [42]. Breast milk is also considered superior to artificial milk in terms of antioxidant and anti-inflammatory properties. Aycicek et al. examined fifty-four healthy-term infants fed breast milk or a cow's milk modified formula and found that oxidative stress markers were lower in the breast milk group [43]. In a study using a human intestinal model, Allan et al. determined that breast milk reduced interleukin-8, a marker of inflammation in the intestinal epithelium, down-regulated toll-like receptor 4 expression, and suppressed inflammatory responses [44]. These reports suggested that the superior antioxidant and anti-inflammatory effects of breast milk compared to formula are due to the higher proportion of whey protein in breast milk. Therefore, changing the protein ratio and increasing the proportion of whey protein over casein protein may strengthen the antioxidant and anti-inflammatory effects. Oxidative stress is a contributing factor to cell damage and the exacerbation of several chronic diseases. Dietary antioxidants aid in fighting against free radicals and thereby prevent or reduce oxidative stress. Corrochano et al. reported that oxidative stress contributes to cell injury and aggravates several chronic diseases, and compared whey from different milk sources and contextualized whey proteins within the broader spectrum of known food antioxidants [45]. However, for whey proteins to be effective in boosting antioxidant levels in target organs, their antioxidant activity must survive not only processing, but also upper gut transit and arrival in the bloodstream. In this study, it was shown that direct cell exposure to whey samples can increase intracellular antioxidants such as glutathione. The physiological relevance of these in vitro assays is questionable, and there is conflicting evidence from dietary intervention trials involving rats and humans that whey products can boost cellular antioxidant biomarkers.

5. Future Directions

We will continue to test whey protein interventions in an LBW, non-obese, hyperglycemic mouse model and obese animal models with high-fat diet challenge to examine its effects on fat weight and insulin resistance. Furthermore, since mice were reared exclusively on either whey protein or casein protein in these experiments, examining mixed interventions in which whey and casein are administered together at different ratios will be necessary. Such mixed formulations must be investigated to apply the interventions in clinical practice.

6. Conclusions

Whey protein intervention started in the fetal period seems to increase the levels of several metabolites with anti-inflammatory and antioxidant effects, leading to reduced fat weight and improved insulin resistance.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/nu15102263/s1. Table S1: principal component score; Table S2: metabolites and principal component score; Table S3: Results of comparative analysis.

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主論文の和文の要約

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博士の専攻分野の名称：博士（医学）

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（雄マウスにおけるホエイタンパク食の体脂肪減少効果について）

1. はじめに

日本は先進国の中で、平均出生体重が減少し、かつ低出生体重児 (low-birth-weight：LBW) 出生率は減少していない国の一つである [1)]。

低出生体重児は、成人期に肥満や 2 型糖尿病などの疾患を発症するリスクが高くなる。

胎児は、子宮内で栄養不足になると、体重増加が遅れるなど環境に適応するために生理的な変化を起こし、出生後に栄養環境が改善された場合、相対的に栄養過多となる。

The developmental origins of health and disease(DOHaD)理論 [2)]では、病気のリスクを十分に理解することで、生涯にわたって病気の発症を防ぐことができると考えられている。小児科医にとって DOHaD 理論は、幼児期から青年期にかけての栄養管理が必要であるという考えを支持している [3)]。

本研究では、低出生体重児の青年期でのメタボリックシンドローム罹患から保護する可能性のある栄養素であるホエイタンパクに注目した。

ホエイタンパクは、ヨーグルトやチーズなどの乳製品に多く含まれるタンパク質で、多くの健康上の利点があると言われており、近年、機能性食品として需要が高まっている [4)]。

また、ホエイタンパクは、母乳や人工乳にも含まれている。母乳中のタンパク質の組成は、授乳期間中に変化する。初乳は 90%のホエイタンパクと 10%のカゼインタンパクで構成されているが、成乳ではその比率が 60%のホエイタンパクと 40%のカゼインタンパクに移行する。一方、牛乳は、通常 20%のホエイタンパクと 80%のカゼインタンパクで構成されている [4)]。

ホエイタンパクの主な成分としては、ラクトフェリン、βラクトグロブリン、αラクトアルブミン、グリコマクロペプチド、免疫グロブリンなどがあり [5)]、ホエイタンパクはカゼインタンパクにくらべて成分表(表 1)の通り様々なタンパク質を含んでいる。カゼインタンパクとホエイタンパクの構成アミノ酸を表 2 に示す。

またホエイタンパクには以下の作用の報告がある。

1：糖代謝効果

ホエイタンパクは、末梢由来のセロトニンの分泌を抑制し、肝臓組織における fibroblast growth factor 21 の発現を抑制し、また食事性糖尿病の発症を予防する [6)]。

2：筋肉合成効果

ホエイタンパクは、筋肉合成に必要な代謝経路に存在する mTOR（mammalian target of rapamycin）を活性化することにより、筋肉合成を促進する [7)]。

3：抗炎症作用

マウス肝炎モデルにおいて、ホエイタンパクが炎症性サイトカインの産生を抑制することで、肝細胞の壊死やアポトーシスを抑制することがわかっている[8]。臨床においても COPD 患者の炎症反応を抑制するという同様の結果を示している[9]。

4：抗酸化作用

ホエイタンパクは、in vitro で抗酸化作用を示した[10]。

5：脂質代謝効果

ホエイタンパクは、脂質代謝に関わる転写因子に影響を与えることで、マウスにおいてトリグリセリドの分解を促進し、脂肪酸の合成を抑制する[11]。

近年、低出生体重児の非肥満型 2 型糖尿病発症が臨床報告されている。

桑原らは、出生後に十分な栄養を摂取して育った低出生体重児は、成人期以降に 2 型糖尿病を発症することが多く、発症時に皮下脂肪に比べて内臓脂肪が有意に蓄積していることを報告した[12]。

また、長野らは、思春期前までは肥満ではなかった低出生体重児が成人期に 2 型糖尿病を発症することが多く、体脂肪は正常範囲にあるが筋肉量は不足していることを明らかにした[13]。

このことから、低出生体重児の 2 型糖尿病の発症には筋肉量と脂質代謝が関与している可能性を示唆される。

早産児は正期産児に比べて、酸化ストレスマーカーが高いことが報告されている[14]。さらに、男性は女性よりも酸化ストレスマーカーのレベルが高く、その結果、男性は 2 型糖尿病や心血管イベントを起こしやすいとも報告されている[15,16]。

ホエイタンパクには、筋肉合成を促進し、糖・脂質代謝を改善する作用があり、さらに抗炎症作用と抗酸化作用もあることから、低出生体重児に乳幼児期、幼児期、思春期前にホエイタンパクを多く含む食事を与えることは、その後の肥満や糖尿病などの発症を予防するのに役立つと考えられる。しかし、ホエイタンパクがこれらの効果を発揮するメカニズムは、まだ完全には解明されていない[17]。

DOHaD 理論では、出生後の環境への適応変化は、胎内に存在するときから始まっている。そのため、ホエイタンパクでの介入は、胎児期からが必要と考える。

そこで本研究では、胎生期から成体期に至るまでホエイタンパクを摂取させた雄マウスを、同期間カゼインタンパク食を摂取させた雄マウスと比較し、身体的および生化学的変化を測定することにより、ホエイタンパクが糖・脂質代謝に及ぼす影響を調べ、体脂肪減少に関与するメカニズムを明らかにする。

2. 材料と方法

2.1. 実験動物

すべての実験計画および手順は、日本大学板橋病院動物実験委員会の承認を得た（承認 ID：

AP20MED018-1、承認日：2020 年 6 月 5 日)。
妊娠 2 日目の ICR 系統の妊娠マウスは、三共ラボサービス株式会社（東京、日本）から購入した。

2.2. 飼育条件
ICR 妊娠マウスは、到着後、カゼインタンパク食を与えるカゼイン群(C 群)とホエイタンパク食を与えるホエイ群(W 群)の 2 群に分けられた。出生後、雄の仔マウスを選択し、母親と同じ飼料で飼育した。カゼイン群では、マウス実験において妊娠・成長期に投与される標準的な飼料である AIN-93G（カゼイン 20%、L-シスチン 0.3%、トウモロコシデンプン 39.7486%、α-トウモロコシデンプン 13.2%、スクロース 10.0%、大豆油 7.0%、セルロースパウダー5.0%、ミネラル 3.5%、ビタミン 1.0%、酒石酸コリン 0.25%、ターシャリーブチルハイドロキノン 0.0014%：エネルギー359kcal）（オリエンタル酵母工業株式会社、東京都、日本）で飼育した [18)]。
ホエイ群では、AIN-93G のカゼイン成分をホエイに置き換えたブレンド飼料でマウスを飼育した。仔マウスは 12 週齢まで飼育した後、身体的および生化学的測定を行った（図 1)。

2.3. 体重測定
出生から 12 週齢まで週に 1 回の体重測定を実施した。

2.4. 血糖値、血清インスリン、インスリン抵抗性(HOMA-IR)
12 週齢の成体雄マウスを 12 時間絶食させ、イソフルラン吸入麻酔下で解剖した。血液は正中切開による心臓穿刺で採取し、血糖、血清インスリン濃度(immunoreactive insulin levels :IRI)、インスリン抵抗指数(HOMA-IR)を測定した。

2.5. 体組成と脂肪重量
体組成はインピーダンス法を用い、体脂肪率(Fat mass：以下 FM)と除脂肪率(Free Fat mass：以下 FFM)を測定した。FM は脂肪、FFM は体水分量とタンパク質量とミネラル量を合計したもので実質臓器と筋肉量と高い相関を示す。脂肪重量は 観察可能な脂肪組織をすべて採取し測定した。

2.6. 血清リポ蛋白濃度
血清リポ蛋白は、ゲル浸透高速液体クロマトグラフィー法（LipoSEARCH®; Skylight Biotech, 秋田、日本）を用いて、コレステロールとトリグリセリドを各々の分画に分離した [19-21)]。

2.7. 肝臓の脂質代謝関連遺伝子発現解析
12 週齢の雄マウスの肝臓を用いて脂質代謝関連遺伝子（Peroxisome proliferator-activating

receptor α ：PPARα、Peroxisome proliferator-activating receptor γ ：PPARγ、Sterol Regulatory Element-Binding Protein-1c：SREBP1c、Hormone sensitive lipase：HSL、Lipoprotein lipase：LPL）の RNA 発現量を、リアルタイム定量ポリメラーゼ連鎖反応（RT-qPCR）を用いて測定した。

2.8. 脂肪組織のメタボローム解析
12 週齢の雄マウスの脂肪組織（各群 n = 5）を用いてメタボローム解析を行った。

2.9. 血清クレアチニンおよび尿クレアチニン
血清クレアチニンは酵素法を用いて測定した。尿クレアチニンは、マウスを実験動物用代謝ケージで 24 時間飼育して尿を採取し、酵素法により測定した。

2.10. 統計学的系解析
データは、平均±平均の標準誤差で表示した。2 郡間の比較は必要に応じて JMP ver.14 を使用して、Mann-Whitney U 検定を行った。p 値＜0.05 を有意差ありとし、0.05＜p＜0.10 の場合はわずかに有意とした。

3 結果
3.1. 体重の推移
出生時の体重は、両群間で有意な差はなかったが、その後、12 週齢まで一貫して W 群のほうが C 群より体重は低値であった。12 週間齢時の体重では、W 群 48.3g、C 群 61.0g と W 群で有意に低値だった(p＜0.01)（図 2a,b)。

3.2. 血糖値、IRI、HOMA-IR
平均空腹時血糖値は、両群間で有意差はなかった(W 群 177.5 vs C 群 184.7 mg/dL、p = 0.75)。IRI は、C 群よりも W 群でわずかに低値だった（W 群 22.0 vs C 群 47.0μIU/mL、p= 0.07)。HOMA-IR は、C 群より W 群で有意に低値だった（W 群 7.9 vs C 群 19.2、p=0.02）（表 1)。

3.3. 脂肪重量と体組成
脂肪重量は、C 群よりも W 群で有意に低値だった（W 群 2.4 vs C 群 3.8g、p < 0.01)。しかし、体組成は、両群とも FFM、FM に有意差はなかった。(W 群 67.9 vs C 群 64.7%、p=0.63)、(W 群 32.0 vs C 群 35.3%、p=0.63）（表 2)。

3.4. 血清および尿中 Cr
血清 Cr 値は、C 群より W 群の方でわずかに高値だった(W 群 0.14 vs C 群 0.11mg/dL、

p=0.06)。また尿中 Cr 濃度は C 群よりも W 群で有意に高値だった(W 群 54.6 vs C 群 35.8mg/dL、p=0.02)（表 3)。

3.5. 血清リポ蛋白質分画

血清コレステロール値については、(総コレステロール、VLDL(very low density lipoprotein)コレステロール、LDL(low density lipoprotein)コレステロール、および HDL(high density lipoprotein)コレステロール)いずれ分画も両群間で有意差は認められなかった。(W 群 173.51 vs C 群 153.46mg/dL、p=0.63; W 群 10.85 vs C 群 10.94 mg/dL、p=0.94; W 群 25.16 vs C 群 23.38 mg/dL、p=0.52; W 群 136.44 vs C 群 116.16 mg/dL、p=0.26)。一方、中性脂肪は、測定された全ての分画で、C 群よりも W 群で有意に低値だった（W 群 51.47 vs C 群 119.2 mg/dL、p=0.01）（表 4)。

3.6. 肝臓の脂質代謝関連遺伝子発現解析

PPARα の発現は C 群よりも W 群でわずかに高値だった（p=0.08)。しかし、評価した他の脂質代謝関連遺伝子(PPARγ, SREBP1c ,HSL, LPL)については、有意差は認められなかった（PPARγ：p= 0.27、SREBP1c：p=0.73、HSL：p=0.58、LPL：p=0.25）（表 5)。

3.7. 脂肪組織のメタボローム解析

抗酸化作用をもつグルタチオン、抗炎症作用をもつ 1-メチルニコチンアミド、糖代謝に関連するミオイノシトールリン酸（1-リン酸・3-リン酸）は、各々W 群で C 群より有意に高値だった（グルタチオン：p＜0.01、1-メチルニコチンアミド：p=0.04、ミオイノシトールリン酸：p=0.01）（表 6)。

4. 考察

本研究では、マウスモデルにおいて、ホエイタンパクの摂取が脂質代謝を活性化し、脂肪量を減少させ、インスリン抵抗性を低下させることが確認された。

これらの結果は、ホエイタンパクの摂取によりβ酸化の促進と、抗炎症作用と抗酸化作用が認められたためと推論している（図 3 a,b)。

4.1. 脂質代謝

血清総トリグリセリド値は C 群に比べて W 群で有意に低値であり、qPCR において W 群は C 群に比べて PPARα の RNA の発現量が高い傾向であることが確認された。

以前の報告では、ホエイタンパクが PPARα の発現を増加させること [11]、PPARα が細胞内のミトコンドリア β 酸化を増加させ、脂質代謝を活性化すること [22]が示されている。従って、ホエイタンパクの摂取は、PPARα の発現を増加させることでトリグリセリドの利用を促進すると推測される。

さらに W 群の脂肪組織は、ニコチンアミドの代謝物である 1-メチルニコチンアミドがカゼ

イン群に比べ有意に高値であった。1-メチルニコチンアミドは抗炎症作用をもち、また肝臓のβ酸化に関与するSIRT1タンパクを安定化させ、β 酸化促進作用を有するため[23]、ホエイタンパクの摂取は β 酸化を調節することで脂質代謝を改善させると仮定される(図3a)。本研究では、コレステロールは両群間で有意差は認めなかったがトリグリセリドでは有意差を認めた。ホエイ群ではトリグリセリドの利用がβ酸化亢進で亢進したため、低下した。本研究では、コレステロール値は変化を認めなかった。

4.2. 糖代謝

本研究では、HOMA-IRがW群でC群に比べて低値であった。マウスでのHOMA-IRの基準値はないため、総合的な評価にはなってしまうが、血清インスリン値としてもW群で低い傾向を認めている。今後、経口ブドウ糖負荷試験などでの追加検討が必要ではあるが、ホエイタンパクを与えることは、インスリン分泌抑制やインスリン抵抗性を抑えるということが考えられた。

脂肪組織では、アディポサイトカインの制御機構が破壊され、炎症性サイトカインが過剰に産生されることでインスリン抵抗性が上昇することが報告されている[24]。

また、酸化ストレスは、脂肪組織のインスリン受容体を減少・不活性化させ、遺伝子の発現や分泌を低下させる[25,27]。

メタボローム解析の結果、脂肪組織中の 1-メチルニコチンアミドとグルタチオンの値は、C群よりもW群で有意に高いことが示された。

ホエイタンパク内に含まれているラクトフェリンが腸内環境改善に寄与することは報告されている[26]。腸内環境の改善が水溶性ビタミンの吸収率を向上させ、ニコチンアミドの代謝物である 1-メチルニコチンアミドの増加を導いた可能性はある。1-メチルニコチンアミドの増加は、脂肪細胞の慢性炎症を抑制し、インスリン抵抗性を改善させると推測される。また、ホエイタンパクはグルタチオンの原料であるシステイン含有量が多いため発現量が増加するという報告がある[27]。グルタチオンは抗酸化作用をもつため[29,30]、ホエイタンパク摂取によるグルタチオンの増加は、脂肪細胞の酸化ストレスを低下させ、インスリン抵抗性を改善させると推測される(図3b)。

4.3. 筋組織によるインスリン抵抗性の改善

今回の研究では、ホエイタンパクが抗炎症マーカーである 1-メチルニコチンアミドを増加させることがわかった。

ホエイタンパクは、大豆が原料である植物性タンパクのソイタンパクに比べて、血中の炎症マーカーであるIL-6とTNF-α 値を低下させ、筋代謝に影響を与えることが報告されている[31]。筋肉量の減少と筋原性インスリン抵抗性の関連性については報告されており[32]、筋肉量の増加はインスリン抵抗性の改善を来す可能性がある。

ホエイタンパクは、クレアチニンを増加させるとともに筋肉量を増加させる栄養補助食品として一般的に使用されている[33]。よって、ホエイタンパクが血清クレアチニンの上昇に

寄与した可能性がある。本研究では筋肉量の測定はしていないが、筋肉量の増加が起きていれば、それもインスリン抵抗性を改善させた要因の一つである可能性がある。

4.4. ミオイノシトールリン酸

メタボローム解析において、脂肪組織のミオイノシトール 1 リン酸および 3 リン酸が、C 群よりも W 群で有意に高いことが判明した。ミオイノシトール 1-リン酸およびミオイノシトール 3-リン酸は、ミオイノシトール代謝経路に属している。ミオイノシトールは、細胞膜リン脂質の構成成分であり、シグナル伝達に関与している。ミオイノシトールの発現が低下したラットでは、肝臓の TG 含有量が高いことが報告されている [34)]。またミオイノシトールはインスリン抵抗性を改善すると報告されている [35)]。ミオイノシトール 1-リン酸および 3-リン酸が直接的に糖や脂質の代謝を改善したという報告はないが、今回の発見はその関連性を示唆するものであると判断した。

4.5. 乳幼児と酸化ストレス

Matsubasa らは、日本の超低出生体重児 50 人の生後数日間の尿を採取し、酸化ストレスマーカーである 8-ヒドロキシデオキシグアノシンを測定した。その結果、超低出生体重児の尿中 8-ヒドロキシデオキシグアノシンは満期産児よりも高く、出生後の体重増加に伴って減少することが示された [36)]。

また、Piyush らは、栄養不良の母親から生まれた small for gestational age (SGA)の乳児が、酸化ストレスマーカーであるマロンジアルデヒドの濃度が高く、スーパーオキシドジスムターゼ、カタラーゼ、グルタチオンペルオキシダーゼなどの抗酸化系の酵素値が、健康な母親から生まれた appropriate for gestational age (ASA)の乳児よりも低いことを報告している [37)]。

これらの結果は、低出生体重児で出生前ストレスが高い乳児は、酸化ストレスが高く、抗酸化能力が低いことを示唆している。

今回の研究では、胎生期からホエイタンパクを摂取させることで、抗酸化能と抗炎症能が向上することが明らかになった。したがって、出生前ストレスが高い低出生体重児にホエイタンパクを摂取させることで、酸化ストレスを軽減し、抗酸化力を向上させることができる可能性がある。

4.6. 母乳と粉ミルクの抗酸化作用と抗炎症作用の比較

母乳は、多くの点で乳児にとって最良の栄養源と考えられている。母乳には、炭水化物、タンパク質、脂肪、ビタミン、ミネラル、消化酵素、ホルモンが含まれている。

母乳のタンパク質組成は、時間の経過とともに変化する子供の成長に適応し、ホエイタンパクとカゼインタンパクの割合が変化する [38)]。

また、母乳は抗酸化作用や抗炎症作用の点でも、人工乳より優れていると考えられている。Aycicek らは、54 人の健康な乳児に母乳と粉ミルクを与えて、酸化ストレスマーカーが母乳群で低下していることを明らかにした[39)]。
また、Allan らは人の腸管モデルを用いた研究にて、母乳が腸管上皮の IL-8 を減少させ、TLR-4 の発現を抑制することで、炎症反応を抑制することを明らかにした[40)]。
これらの報告から、母乳の優れた抗酸化作用や抗炎症作用は、母乳に含まれるホエイタンパクの割合が高いためであると推測される。したがって、タンパク質の比率を変え、カゼインタンパクよりもホエイタンパクの比率を高めることで、抗酸化作用や抗炎症作用が強化されると考えられる。
酸化ストレスは、細胞の損傷やいくつかの慢性疾患の悪化の一因となる。食事から摂取する抗酸化物質は、活性酸素に対抗し、酸化ストレスを予防・軽減する。Corrochano らは、酸化ストレスが細胞傷害の一因となり、いくつかの慢性疾患を悪化させることを報告し、様々なホエイを比較した結果、ホエイタンパクを食品抗酸化物質の一つに位置づけた。[41)]。

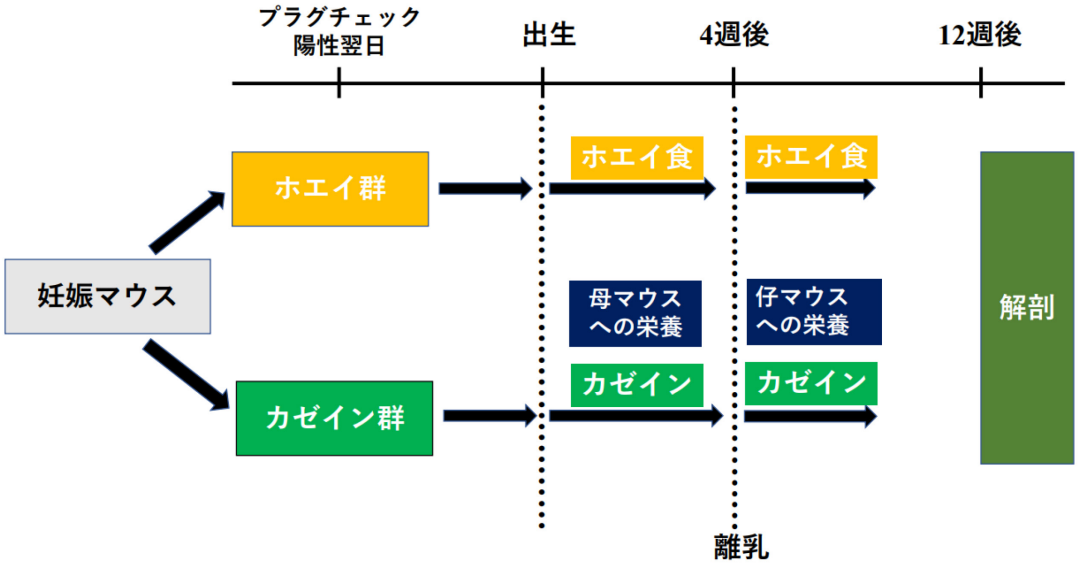
5. 今後の展望

ホエイタンパクの脂肪重量とインスリン抵抗性に対する効果を調べるために、低出生体重児モデルマウスと高脂肪食を与えた肥満モデルマウスで、ホエイプロテインの介入実験を行う。
さらに、今回の実験ではタンパク質の成分としてホエイタンパクまたはカゼインタンパクのどちらか一方のみで飼育されていたため、今後、臨床に応用するためにホエイタンパクとカゼインタンパクを異なる比率で併用投与する介入実験を検討する。

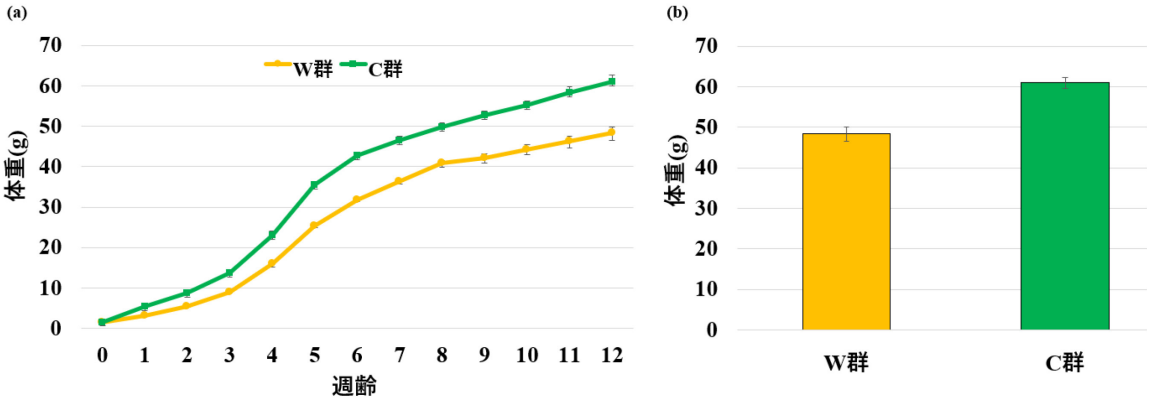
6. 結論

胎生期に開始したホエイタンパクの介入は、抗炎症および抗酸化作用を有するいくつかの代謝産物を増加させた。それにより、脂肪重量は減少し、インスリン抵抗性は改善された。

（図１）


プラグチェック
陽性翌日
出生
4週後
12週後
ホエイ群
カゼイン群
妊娠マウス
ホエイ食
ホエイ食
母マウス
への栄養
仔マウス
への栄養
カゼイン
カゼイン
解剖
離乳

（図２）


(a)
W群
C群
体重(g)
週齢
(b)
体重(g)
W群
C群

（図 3）

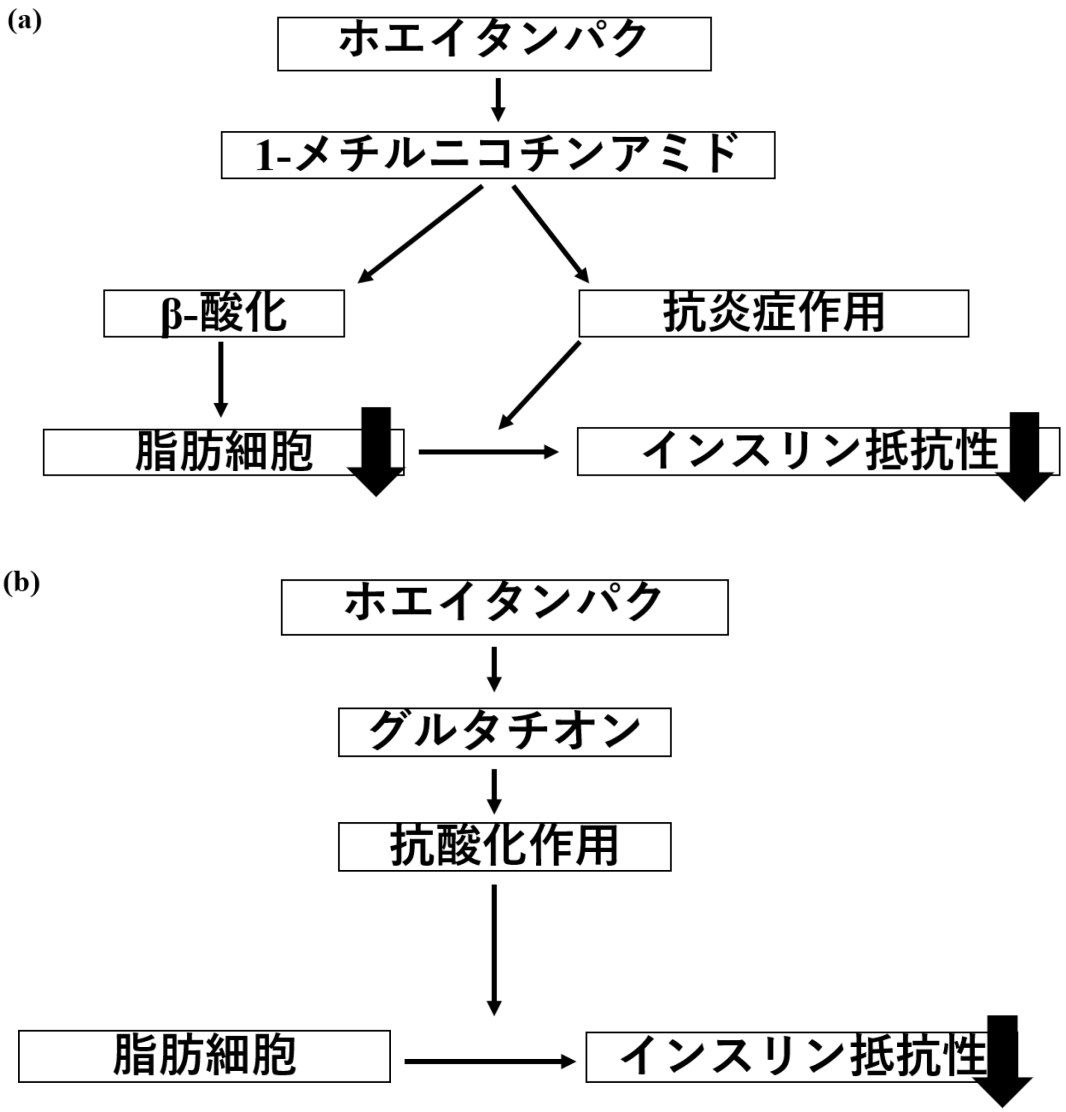

(a)
ホエイタンパク
1-メチルニコチンアミド
β-酸化
抗炎症作用
脂肪細胞
インスリン抵抗性
(b)
ホエイタンパク
グルタチオン
抗酸化作用
脂肪細胞
インスリン抵抗性

表1

	成分	%
カゼイン	α_{s1}-カゼイン	38
	α_{s2}-カゼイン	10
	β-カゼイン	38
	κ-カゼイン	13
ホエイ	βラクトグロブリン	50
	αラクトアルブミン	23
	ウシ血清アルブミン	8
	その他	19
	乳塩基性タンパク質(MBP)	1

表2

アミノ酸	含有(g/100・蛋白質)	
	カゼイン	ホエイ
トリプトファン	1.4	2.1
フェニルアラニン	5.1	3.8
ロイシン	10.4	11.1
イソロイシン	5.7	6.8
スレオニン	4.6	8
メチオニン	2.8	2.4
リシン	8.3	9.9
バリン	6.8	6.8
ヒスチジン	2.9	2.2
アルギニン	4	3
システイン	0.3	2.4
プロリン	11.2	5.2
アラニン	3.1	5
アスパラギン酸	7.3	11.3
セリン	5.8	5.2
グルタミン酸	23	19.2
グリシン	2.1	2.2
チロシン	6	3.5

表 3　血糖値、IRI、HOMA-IR

	W 群(n=6)	C 群(n=6)	*p-Value*
血糖値（㎎/dL）	177.5.0±19.0	184.7±15.9	0.75
IRI（μIU/mL）	22.0±11.7	47.0±17.4	0.07
HOMA-IR	7.9±3.1	19.2±5.5	0.02

Data are shown as the mean ± standard error of the mean (n = 6 per group)

HOMA-IR: homeostasis model assessment of insulin resistance、

IRI: immunoreactive insulin

表 4　体重と脂肪重量と体組成

	W 群(n=6)	C 群(n=6)	*p-Value*
体重(g)	48.3±1.7	61.0±1.4	<0.01
脂肪重量（g）	2.4±0.2	3.8±0.4	<0.01
FFM (%)	67.9±2.9	64.7±3.9	0.63
FM (%)	32.0±2.9	35.26±3.9	0.63

Data are shown as the mean ± standard error of the mean (n = 6 per group)

FFM: Fat free mass, FM: Fat mass

表 5　血清および尿中 Cr

	W 群(n=6)	C 群(n=6)	*p-Value*
血清 Cr（mg/dL）	0.14±0.01	0.11±0.01	0.06
尿中 Cr（mg/dL）	54.6±5.7	35.8±2.9	0.02

Data are shown as the mean ± standard error of the mean (n = 6 per group)

表 6　血清リポ蛋白質分画

	W 群(n=6)	C 群(n=6)	*p-Value*
総コレステロール（mg/dL）	173.51±15.6	153.46±11.9	0.63
VLDL コレステロール（mg/dL）	10.85±0.6	10.94±2.2	0.94
LDL コレステロール（mg/dL）	25.16±3.1	23.38±4.8	0.52
HDL コレステロール（mg/dL）	136.44±13.3	116.16±9.2	0.26
中性脂肪（mg/dL）	51.47±5.5	119.2±35.0	0.01

Data are shown as the mean ± standard error of the mean (n = 6 per group)

VLDL: very low density lipoprotein、LDL: low density lipoprotein、HDL: high density lipoprotein

表 7　肝臓の脂質代謝関連遺伝子発現解析

	W 群 vs. C 群	
	比*	*p*-Value
PPARα	2.3	0.08
PPARγ	0.17	0.27
SREBP1c	0.9	0.73
HSL	0.9	0.58
LPL	0.6	0.25

(n = 6 per group)　　*2 群間の平均値の比

PPARα：Peroxisome proliferator-activating receptor α (細胞内のミトコンドリア β 酸化を増加させ、脂質代謝を活性化する)、PPARγ：Peroxisome proliferator-activating receptor γ (脂肪前駆細胞から脂肪細胞へと分化させる)、SREBP1c：Sterol Regulatory Element-Binding Protein-1c(脂肪酸やトリグリセリドの合成を促進する)、HSL：Hormone sensitive lipase(細胞内の TG を脂肪酸とグリセロールに分解し、β酸化を促す)、LPL：Lipoprotein lipase(血液中のリポタンパク質を分解し、脂肪酸を細胞内に取り込む)

表 8　脂肪組織のメタボローム解析

		W 群 vs. C 群	
		比*	*p*-Value
抗酸化	アスコルビン酸	1.1	0.775
	カルノシン	22	0.323
	グルタチオン	7.1	0.004
	ヒポタウリン	29	0.286
	酒石酸	0.6	0.458
抗炎症	1－メチルニコチンアミド	2	0.044
	ヒスチジン	1.6	0.243
糖代謝	ミオイノシトール 1 リン酸・3 リン酸	3.1	0.013

(n = 5 per group)　　*2 群間の平均値の比

メタボローム解析　そのほかの結果

1.

Supplementary Table S1. Principal component score

Contribution rate (%)		Whey					Casein				
		whey2	whey3	whey4	whey5	whey6	casein2	casein3	casein4	casein5	casein6
PC1	54.74	-11.31	10.39	-36.93	2.29	6.28	7.13	3.04	7.02	5.30	6.79
PC2	11.92	17.19	-0.89	-7.06	2.83	1.27	-1.63	-4.71	-3.16	-1.71	-2.12
PC3	9.84	-3.20	9.10	2.46	4.77	6.72	0.03	-7.20	-1.95	-10.28	-0.46
PC4	5.86	1.19	-0.03	-0.10	-1.21	-5.56	11.52	0.65	0.39	-5.03	-1.82
PC5	4.49	-0.23	4.11	1.14	-1.94	-3.33	1.86	-8.04	-1.41	6.76	1.07
PC6	4.03	0.06	-4.35	-0.01	-0.59	0.10	0.62	-2.66	-0.67	-2.54	10.04
PC7	3.58	-2.00	-5.54	0.07	3.28	5.19	4.12	-2.10	-3.32	2.87	-2.56
PC8	3.26	0.36	-2.56	0.25	-2.13	2.06	0.04	-3.97	8.58	-0.72	-1.91
PC9	2.27	-1.03	-1.12	-0.24	6.97	-4.27	-1.21	-1.06	1.98	0.08	-0.08

PC, principal component

2.

Supplementary Table S2. Metabolites and principal component score

	Compound name	PubChem CID	HMDB ID	m/z	MT/RT	PC1	PC2
A_0003	Crotonic acid	637090		85.029	9.15	1.7E-01	4.0E-01
A_0005	Butyric acid Isobutyric acid	264 6590	HMDB0000039 HMDB0001873	87.045	8.43	-8.2E-01	-2.8E-02
A_0006	Lactic acid	612	HMDB0000190,HMDB0001311	89.024	9.14	-9.7E-01	-2.3E-01
A_0007	Isovaleric acid DL-2-Methylbutyric Acid Valeric acid	10430 8314 7991	HMDB0000718 HMDB0002176 HMDB0000892	101.061	7.95	-8.6E-01	3.0E-01
A_0008	3-Hydroxybutyric acid	441	HMDB0000011,HMDB0000357,HMDB0000442	103.040	8.16	-2.4E-01	-1.4E-01
A_0009	2-Hydroxybutyric acid	440864	HMDB0000008	103.040	8.36	-2.6E-01	-3.6E-01
A_0010	2-Hydroxyisobutyric acid	11671	HMDB0000729	103.040	8.45	-9.7E-01	-1.3E-01
A_0011	Glyceric acid	439194	HMDB0000139,HMDB0006372	105.019	8.81	-7.7E-01	-4.7E-01
A_0012	Fumaric acid	444972	HMDB0000134	115.003	17.83	-8.9E-01	1.6E-01
A_0014	Hexanoic acid	8892	HMDB0000535	115.076	7.66	-6.8E-01	1.5E-01
A_0015	*N*-Acetylglycine	10972	HMDB0000532	116.035	8.14	-7.4E-01	4.1E-01
A_0016	Succinic acid	1110	HMDB0000254	117.019	15.74	-9.1E-01	-3.2E-01
A_0017	β-Hydroxyisovaleric acid	69362	HMDB0000754	117.055	7.84	-5.5E-01	1.3E-01
A_0018	2-Hydroxyvaleric acid	98009	HMDB0001863	117.056	7.94	-9.7E-01	1.4E-01
A_0019	Isethionic acid	7866	HMDB0003903	124.991	9.70	-9.8E-01	-1.0E-01
A_0020	5-Oxoproline	7405	HMDB0000267	128.035	8.10	-5.4E-01	1.8E-01
A_0021	5-Oxohexanoic acid	18407		129.056	7.79	1.7E-01	-1.1E-01
A_0022	4-Methyl-2-oxovaleric acid 3-Methyl-2-oxovaleric acid 2-Oxohexanoic acid	70 47 159664	HMDB0000695 HMDB0000491 HMDB0001864	129.056	8.18	-7.0E-01	-3.8E-01
A_0023	Heptanoic acid	8094	HMDB0000666	129.092	7.41	-4.1E-01	1.3E-01
A_0024	*N*-Acetylalanine	88064	HMDB0000766	130.051	7.61	1.5E-01	5.4E-01
A_0025	6-Hydroxyhexanoic acid	14490		131.071	7.29	-9.1E-01	3.6E-01
A_0026	2-Hydroxy-4-methylvaleric acid	439960	HMDB0000624	131.072	7.59	-9.5E-01	2.0E-01
A_0027	Malic acid	525	HMDB0000156,HMDB0000744	133.014	15.97	-8.4E-01	2.8E-01
A_0028	Threonic acid	5460407	HMDB0000943	135.030	7.90	-8.8E-01	-2.9E-01
A_0029	6-Hydroxynicotinic acid	72924	HMDB0002658	138.019	8.04	3.3E-01	-2.2E-01
A_0030	Ethanolamine phosphate	1015	HMDB0000224	140.012	6.89	-9.9E-01	-1.1E-01
A_0031	*N*-Ethylmaleimide_+H_2O	4362		142.051	7.47	-1.2E-02	3.5E-01
A_0032	Octanoic acid	379	HMDB0000482	143.107	7.20	-5.1E-01	6.3E-01
A_0033	XA0004			144.031	7.97	-9.3E-01	3.1E-01
A_0034	4-Acetamidobutanoic acid	18189	HMDB0003681	144.066	7.39	-2.8E-01	2.2E-01
A_0035	2-Oxoglutaric acid	51	HMDB0000208	145.013	15.92	-9.1E-01	-3.7E-01
A_0036	2-Hydroxyglutaric acid	43	HMDB0000606,HMDB0000694	147.029	13.51	-7.4E-01	-3.4E-01
A_0037	Tartaric acid	444305	HMDB0000956	149.009	16.39	-2.7E-01	-2.3E-01
A_0038	3-Phenylpropionic acid	107	HMDB0000764	149.060	7.56	-1.4E-01	7.3E-01
A_0039	Cysteinesulfinic acid	1549098	HMDB0000996	152.003	8.15	-9.4E-02	6.1E-01
A_0040	Orotic acid	967	HMDB0000226	155.010	8.41	-9.7E-01	-1.7E-01
A_0041	Dihydroorotic acid	439216	HMDB0000528	157.025	8.00	-9.1E-01	-3.7E-01
A_0042	2-Oxooctanoic acid	67600		157.087	7.33	1.7E-01	2.8E-02
A_0043	Pelargonic acid	8158	HMDB0000847	157.123	7.02	-6.4E-01	1.8E-01
A_0044	8-Hydroxyoctanoic acid	69820		159.103	6.94	4.3E-01	-6.0E-02
A_0045	2-Hydroxyoctanoic acid	94180	HMDB0000711	159.103	7.01	-8.2E-01	-3.1E-01
A_0046	3-Hydroxy-3-methylglutaric acid	1662		161.045	12.60	-2.8E-01	9.1E-01
A_0047	*N*-Acetylcysteine	12035	HMDB0001890	162.024	7.54	-2.0E-01	6.5E-01
A_0048	o-Coumaric acid *p*-Coumaric acid	637540 637542	HMDB0002641 HMDB0002035	163.039	7.46	1.8E-01	-8.6E-02
A_0049	4-Hydroxyphenylglyoxylic acid	355		165.019	11.08	-9.1E-01	-3.7E-01
A_0050	Terephthalic acid	7489	HMDB0002428	165.019	13.29	7.1E-01	-1.3E-01
A_0051	Perillic acid	1256	HMDB0004586	165.091	7.02	-4.0E-01	-2.6E-01
A_0052	XA0012			166.018	8.02	-9.4E-01	-9.3E-02
A_0053	Phosphoenolpyruvic acid	1005	HMDB0000263	166.974	15.83	-5.4E-01	5.9E-01
A_0054	Uric acid	1175	HMDB0000289	167.021	7.62	-7.1E-01	-3.7E-02
A_0055	Homogentisic acid *p*-Hydroxymandelic acid	780 328	HMDB0000130 HMDB0000822	167.035	7.38	-9.7E-01	-2.0E-01
A_0056	Dihydroxyacetone phosphate	668	HMDB0001473	168.990	10.61	-9.8E-01	6.9E-02
A_0058	Glycerol 2-phosphate	2526		171.006	10.44	-9.1E-01	-3.7E-01
A_0059	Glycerol 3-phosphate	439162	HMDB0000126	171.006	10.16	-9.5E-01	3.1E-01
A_0060	Decanoic acid	2969	HMDB0000511	171.139	6.87	-3.7E-01	3.7E-01
A_0061	Isovalerylalanine *N*-Acetylleucine	129285 70912	HMDB0000747 HMDB0011756	172.098	6.92	-2.3E-01	3.2E-01
A_0062	cis-Aconitic acid	643757	HMDB0000072	173.009	18.72	-9.3E-01	-2.1E-01
A_0063	Suberic acid	10457	HMDB0000893	173.082	10.74	5.3E-01	-2.4E-01
A_0064	*N*-Acetylaspartic acid	65065	HMDB0000812	174.041	11.95	-6.2E-01	-3.1E-01
A_0065	Ascorbic acid	54670067	HMDB0000044	175.024	7.28	-2.2E-01	1.6E-01

A_0066	*N*-Carbamoylaspartic acid	93072	HMDB0000828	175.035	12.50	-9.1E-01	-3.7E-01
A_0067	Homovanillic acid Hydroxyphenyllactic acid	1738 9378	HMDB0000118 HMDB0000755	181.050	7.11	-9.6E-01	8.5E-02
A_0068	Homocysteic acid	177491	HMDB0002205	182.013	8.02	2.6E-01	-4.7E-02
A_0069	*O*-Phosphoserine	68841	HMDB0000272	184.001	10.30	-9.7E-02	6.6E-01
A_0070	2-Phosphoglyceric acid	439278	HMDB0003391	184.985	14.76	-5.6E-01	5.1E-01
A_0071	3-Phosphoglyceric acid	439183	HMDB0000807	184.986	15.04	-5.4E-01	5.9E-01
A_0072	Undecanoic acid	8180	HMDB0000947	185.154	6.73	-1.5E-01	1.3E-01
A_0073	XA0017			186.114	6.85	-4.6E-01	3.6E-01
A_0074	*N*-Acetylglutamine	25561	HMDB0006029	187.073	6.96	-9.1E-01	-3.7E-01
A_0075	Azelaic acid	2266	HMDB0000784	187.098	10.23	3.7E-01	-1.3E-01
A_0076	10-Hydroxydecanoic acid	74300		187.134	6.67	-8.0E-01	-4.2E-01
A_0077	Kynurenic acid	3845	HMDB0000715	188.036	7.35	-9.7E-01	-1.6E-01
A_0078	*N*-Acetylglutamic acid	70914	HMDB0001138	188.056	10.99	-8.3E-01	-2.0E-01
A_0079	*N*-Acetylmethionine	448580	HMDB0011745	190.054	6.99	-5.7E-01	4.6E-01
A_0080	Isocitric acid	1198	HMDB0000193	191.019	19.08	-3.6E-01	-5.0E-01
A_0081	Citric acid	311	HMDB0000094	191.020	18.16	-9.7E-01	-1.8E-01
A_0082	XA0019			191.020	7.16	-9.8E-01	-2.1E-02
A_0083	Quinic acid	6508	HMDB0003072	191.056	6.97	1.8E-01	2.3E-01
A_0084	Phenaceturic acid	68144	HMDB0000821	192.066	7.06	-5.3E-01	2.2E-01
A_0085	Galacturonic acid Glucuronic acid	439215 94715	HMDB0002545 HMDB0000127	193.035	6.97	-5.0E-01	1.0E-01
A_0086	Gluconic acid	10690	HMDB0000625	195.051	7.05	-9.3E-01	1.7E-02
A_0088	Lauric acid	3893	HMDB0000638	199.171	6.62	-4.3E-01	4.1E-01
A_0089	Sebacic acid	5192	HMDB0000792	201.112	9.81	1.7E-01	-1.1E-01
A_0090	Xanthurenic acid	5699	HMDB0000881	204.029	10.29	1.3E-01	-9.0E-02
A_0091	Indole-3-lactic acid 5-Methoxyindoleacetic acid	676157 18986	HMDB0000671 HMDB0004096	204.066	7.06	-9.8E-01	4.7E-02
A_0092	Mucic acid	3037582	HMDB0000639	209.030	11.60	-9.6E-01	-1.2E-01
A_0093	Phosphocreatine	9548602	HMDB0001511	210.028	10.26	-1.6E-01	-2.8E-01
A_0094	3-Indoxylsulfuric acid	10258	HMDB0000682	212.002	8.14	6.5E-02	1.2E-01
A_0095	Tridecanoic acid	12530	HMDB0000910	213.185	6.50	-6.8E-01	2.4E-01
A_0096	Pantothenic acid	6613	HMDB0000210	218.103	6.61	-8.3E-01	3.3E-01
A_0097	Myristoleic acid	5281119	HMDB0002000	225.186	6.46	-4.4E-01	2.5E-01
A_0098	Myristic acid	11005	HMDB0000806	227.201	6.44	-8.1E-01	9.0E-02
A_0099	Ribulose 5-phosphate	439184	HMDB0000618	229.011	9.29	-5.9E-01	7.0E-01
A_0100	Ribose 5-phosphate	439167	HMDB0001548	229.011	8.95	-2.2E-01	8.9E-01
A_0101	XA0033			242.080	6.57	-9.4E-01	3.3E-01
A_0102	XA0080	440992		243.027	8.65	-9.0E-01	-3.7E-01
A_0103	γ-Glu-Taurine	68759	HMDB0004195	253.051	6.98	-9.7E-01	1.9E-01
A_0104	Ascorbate 2-sulfate	54676864		254.982	11.79	-6.8E-01	5.8E-02
A_0105	XA0035			254.982	11.28	-4.7E-02	1.1E-01
A_0106	Glucose 1-phosphate	65533	HMDB0001586	259.022	8.62	-9.2E-01	3.4E-01
A_0107	Fructose 6-phosphate	603	HMDB0000124	259.022	8.48	-8.5E-01	4.9E-01
A_0108	*myo*-Inositol 1-phosphate *myo*-Inositol 3-phosphate	107737 440194	HMDB0000213 HMDB0006814	259.022	8.75	-4.4E-01	3.1E-01
A_0109	*myo*-Inositol 2-phosphate	160886		259.022	8.94	-4.8E-02	8.4E-01
A_0110	Glucose 6-phosphate	5958	HMDB0001401	259.023	8.39	-7.1E-01	6.7E-01
A_0111	2,3-Diphosphoglyceric acid	186004	HMDB0001294	264.950	14.41	-2.2E-02	1.9E-01
A_0112	6-Phosphogluconic acid	91493	HMDB0001316	275.016	12.02	-5.8E-01	7.6E-01
A_0113	Xanthosine	64959	HMDB0000299	283.066	6.56	-7.6E-01	6.1E-01
A_0114	Orotidine	92751	HMDB0000788	287.051	6.80	-9.7E-01	-1.8E-01
A_0115	Sedoheptulose 7-phosphate	165007	HMDB0001068	289.032	8.21	-5.5E-01	7.4E-01
A_0116	Retinoic acid	444795	HMDB0001852	299.202	6.24	-7.7E-02	7.2E-01
A_0117	*N*-Acetylglucosamine 1-phosphate	440272	HMDB0001367	300.048	8.16	-9.3E-01	3.2E-01
A_0118	*N*-Acetylglucosamine 6-phosphate	440996	HMDB0001062	300.048	7.84	-6.5E-01	6.5E-01
A_0119	cCMP 2',3'-cCMP	19236 68934	HMDB0011691	304.035	6.56	7.5E-02	-2.5E-01
A_0120	*N*-Acetylneuraminic acid	439197	HMDB0000230	308.099	6.22	-9.9E-01	-1.5E-02
A_0121	Ribulose 1,5-diphosphate	123658		308.980	12.65	-3.3E-01	7.6E-01
A_0123	3'-CMP	66535		322.044	8.34	1.7E-01	-2.9E-01
A_0124	CMP	6131	HMDB0000095	322.044	8.17	-7.9E-01	-5.5E-01
A_0125	UMP	6030	HMDB0000288	323.026	8.33	-5.9E-01	-7.0E-01
A_0126	*N*-Glycolylneuraminic acid	440001	HMDB0000833	324.093	6.20	2.3E-01	-2.3E-01
A_0127	cAMP	6076	HMDB0000058	328.045	6.46	-9.1E-01	-3.7E-01
A_0128	Fructose 1,6-diphosphate	172313	HMDB0001058	338.990	11.93	-9.8E-01	9.3E-02
A_0130	AMP	6083	HMDB0000045	346.054	7.91	-7.0E-01	-6.0E-01
A_0131	3'-AMP	41211	HMDB0003540	346.054	8.26	1.6E-01	5.8E-03
A_0132	IMP	8582	HMDB0000175	347.038	8.12	-8.1E-01	-1.6E-01
A_0133	Prostaglandin E_2	5280360	HMDB0001220	351.219	6.02	-8.8E-01	4.6E-01
A_0134	Prostaglandin $F_{2\alpha}$	5280363	HMDB0001139	353.234	5.98	-8.2E-01	5.5E-01

A_0135	GMP	6804	HMDB0001397	362.049	7.81	-5.0E-01	-6.9E-01
A_0136	XA0055			368.999	11.74	-8.0E-01	5.0E-01
A_0137	NADPH_divalent	5884	HMDB0000221	371.539	9.49	-9.8E-01	-1.6E-02
A_0138	CoA_divalent	87642	HMDB0001423	382.548	8.98	1.1E-01	1.1E-01
A_0139	PRPP	7339	HMDB0000280	388.945	13.19	-9.1E-01	-3.7E-01
A_0140	FAD_divalent	643975	HMDB0001248	391.570	6.77	-9.7E-01	-8.6E-02
A_0142	CDP	6132	HMDB0001546	402.012	9.61	-9.1E-01	-3.7E-01
A_0143	UDP	6031	HMDB0000295	402.994	9.75	-9.4E-01	-3.2E-01
A_0144	Acetyl CoA_divalent	444493	HMDB0001206	403.552	8.70	-9.1E-01	-3.7E-01
A_0145	Cholic acid	221493	HMDB0000619	407.280	5.95	3.2E-01	-8.3E-02
A_0146	Thiamine diphosphate	1132	HMDB0001372	423.031	6.83	-8.8E-01	-4.4E-01
A_0148	ADP	6022	HMDB0001341	426.022	9.16	-9.3E-01	-3.2E-01
A_0149	3',5'-ADP	159296	HMDB0000061	426.022	11.02	-4.0E-01	1.4E-01
A_0150	GDP	8977	HMDB0001201	442.017	8.96	-9.5E-01	-2.3E-01
A_0151	XA0065			445.053	6.01	-9.8E-01	-6.8E-02
A_0152	Adenylosuccinic acid	447145	HMDB0000536	462.068	11.34	-2.9E-01	-3.0E-01
A_0155	CTP	6176	HMDB0000082	481.979	10.27	-9.1E-01	-3.7E-01
A_0156	UTP	6133	HMDB0000285	482.961	10.43	-9.3E-01	-3.3E-01
A_0157	CDP-choline	13804	HMDB0001413	487.100	5.88	-9.3E-01	-9.2E-02
A_0159	ATP	5957	HMDB0000538	505.990	9.82	-9.2E-01	-3.4E-01
A_0160	Taurocholic acid	46783527	HMDB0000036	514.287	5.83	2.0E-01	5.1E-02
A_0161	GTP	6830	HMDB0001273	521.988	9.60	-9.6E-01	-2.4E-01
A_0162	ADP-ribose	445794	HMDB0001178	558.064	7.28	2.5E-01	-4.1E-01
A_0163	UDP-galactose UDP-glucose	23724458 8629	HMDB0000302 HMDB0000286	565.053	7.39	-9.8E-01	-3.0E-03
A_0164	UDP-glucuronic acid	17473	HMDB0000935	579.024	9.30	-9.0E-01	-1.5E-01
A_0165	GDP-fucose ADP-glucose	10918995 16500	HMDB0001095 HMDB0006557	588.079	7.14	-6.9E-01	-4.0E-01
A_0166	NAD^+	5893	HMDB0000902	662.099	5.67	-9.3E-01	-3.3E-01
A_0167	$NADP^+$	5886	HMDB0000217	742.064	7.98	-1.0E+00	4.4E-02
C_0001	Urea	1176	HMDB0000294	61.040	18.03	-9.3E-01	-8.7E-02
C_0002	Ethanolamine	700	HMDB0000149	62.060	5.19	-9.1E-01	-1.9E-03
C_0003	3-Aminopropionitrile	1647	HMDB0004101	71.060	5.21	2.6E-01	-4.7E-02
C_0004	XC0001			72.081	5.22	-2.8E-01	9.1E-01
C_0005	Aminoacetone	215	HMDB0002134	74.060	5.61	-9.1E-01	-3.7E-01
C_0006	Gly	750	HMDB0000123	76.039	6.85	-9.8E-01	2.0E-01
C_0007	Trimethylamine *N*-oxide	1145	HMDB0000925	76.076	5.40	-9.6E-01	-2.5E-01
C_0008	Morpholine	8083	HMDB0031581	88.076	5.43	-7.2E-01	2.3E-01
C_0009	Putrescine	1045	HMDB0001414	89.107	3.84	-9.3E-01	2.6E-01
C_0010	β-Ala	239	HMDB0000056	90.055	6.01	-9.7E-01	-1.9E-01
C_0011	Ala	602	HMDB0000161,HMDB0001310	90.055	7.42	-7.4E-01	-8.2E-02
C_0012	Sarcosine	1088	HMDB0000271	90.055	7.81	-9.4E-01	-2.8E-01
C_0013	Dimethylaminoethanol	7902	HMDB0032231	90.091	5.66	-8.8E-01	-2.4E-01
C_0014	Glycerol	753	HMDB0000131	93.055	18.85	2.6E-01	2.9E-01
C_0015	Phenol	996	HMDB0000228	95.047	4.41	-9.2E-01	-1.9E-01
C_0016	Cyclohexylamine	7965		100.112	6.26	-5.6E-01	2.6E-02
C_0017	Acetoacetamide	80077		102.055	18.87	1.8E-01	-1.2E-01
C_0018	Homoserinelactone	73509		102.056	5.78	-9.1E-01	-3.7E-01
C_0019	Cadaverine	273	HMDB0002322	103.123	4.06	-6.6E-01	5.4E-01
C_0020	*N,N*-Dimethylglycine	673	HMDB0000092	104.071	9.03	-7.8E-01	-4.6E-02
C_0021	GABA	119	HMDB0000112	104.071	6.30	-9.2E-01	-3.6E-01
C_0022	2-Aminoisobutyric acid 2-Aminobutyric acid	6119 6657	HMDB0001906 HMDB0000452	104.071	7.91	-6.2E-02	-4.5E-01
C_0023	3-Aminobutyric acid	10932		104.071	6.50	2.0E-01	-1.6E-01
C_0024	Choline	305	HMDB0000097	105.110	5.61	-9.8E-01	1.4E-01
C_0025	Ser	617	HMDB0000187,HMDB0003406	106.050	8.26	-9.8E-01	1.5E-01
C_0026	Diethanolamine	8113	HMDB0004437	106.086	6.25	1.3E-01	9.2E-02
C_0027	Hypotaurine	107812	HMDB0000965	110.027	14.99	-9.6E-01	-2.3E-01
C_0029	Histamine	774	HMDB0000870	112.086	3.90	-8.6E-01	-7.6E-03
C_0030	Uracil	1174	HMDB0000300	113.034	18.86	-5.1E-01	7.7E-01
C_0031	Creatinine	588	HMDB0000562	114.066	5.97	-7.3E-01	-1.8E-01
C_0032	3-Amino-2-piperidone	5200225	HMDB0000323	115.086	6.21	1.7E-02	2.5E-02
C_0033	Pro	614	HMDB0000162,HMDB0003411	116.070	8.90	-9.8E-01	-5.9E-02
C_0034	Guanidoacetic acid	763	HMDB0000128	118.061	6.75	2.2E-02	-3.6E-01
C_0035	Val	1182	HMDB0000883	118.086	8.21	-9.7E-01	1.5E-01
C_0036	Betaine	247	HMDB0000043	118.086	9.29	-9.9E-01	-4.4E-02
C_0037	5-Aminovaleric acid	138	HMDB0003355	118.086	6.58	-3.9E-01	5.3E-01
C_0038	Thr	6288	HMDB0000167	120.065	8.68	-9.9E-01	-7.1E-02
C_0039	Homoserine	12647	HMDB0000719	120.065	8.31	-9.0E-01	8.1E-02
C_0040	Betaine aldehyde_+H_2O	249	HMDB0001252	120.102	6.09	-4.2E-01	6.4E-01
C_0041	4-Hydroxyphenethyl alcohol_-H_2O	10393	HMDB0004284	121.065	18.91	-6.3E-01	-1.6E-01

C_0042	Anserine_divalent	112072	HMDB0000194	121.069	5.57	-9.5E-01	-2.5E-01
C_0043	Cys	594	HMDB0000574,HMDB0003417	122.028	9.35	-6.3E-01	2.8E-01
C_0044	2-Amino-2-(hydroxymethyl)-1,3-propanediol	6503		122.081	6.77	1.3E-01	-9.0E-02
C_0045	Nicotinamide	936	HMDB0001406	123.055	6.08	-8.5E-01	4.2E-01
C_0046	Nicotinic acid	938	HMDB0001488	124.039	8.27	1.8E-01	-8.6E-02
C_0047	Taurine	1123	HMDB0000251	126.022	18.84	-9.8E-01	-8.7E-02
C_0048	1-Methylhistamine	3614	HMDB0000898	126.103	4.01	-5.1E-01	8.2E-01
C_0049	3-Hydroxy-2-methyl-4-pyrone	8369	HMDB0030776	127.038	18.92	1.1E-01	-5.9E-01
C_0051	Imidazole-4-acetic acid	96215	HMDB0002024	127.050	6.57	-4.0E-01	8.3E-01
C_0052	XC0016			129.066	7.17	-7.8E-01	-1.8E-01
C_0053	4-Oxopyrrolidine-2-carboxylic acid	107541		130.050	9.05	-9.6E-01	-8.2E-03
C_0054	Pipecolic acid	439227	HMDB0000070,HMDB0000716,HMDB0005960	130.087	8.43	-9.3E-01	-3.4E-01
C_0055	*trans*-Glutaconic acid	5280498	HMDB0000620	131.034	19.62	-8.3E-01	-3.2E-01
C_0056	*N*-Acetylputrescine	122356	HMDB0002064	131.118	6.90	-1.5E-01	4.9E-01
C_0057	Hydroxyproline	5810	HMDB0000725	132.066	9.94	-9.8E-01	-1.2E-01
C_0058	3-Guanidinopropionic acid	67701		132.076	6.56	-8.1E-01	-2.1E-01
C_0059	6-Aminohexanoic acid	564	HMDB0001901	132.102	6.77	-9.8E-01	1.3E-01
C_0060	Leu	857	HMDB0000687	132.102	8.47	-9.5E-01	3.0E-01
C_0061	Ile	791	HMDB0000172	132.102	8.37	-9.5E-01	2.7E-01
C_0062	Gly-Gly	11163	HMDB0011733	133.060	6.85	-4.4E-01	-2.9E-02
C_0063	Asn	236	HMDB0000168,HMDB0033780	133.060	8.69	-9.9E-01	4.1E-03
C_0064	Creatine	586	HMDB0000064	133.079	7.24	-9.7E-01	-1.6E-01
C_0065	Ornithine	389	HMDB0000214,HMDB0003374	133.097	5.57	-4.6E-01	5.1E-01
C_0066	Thiaproline	9934		134.027	11.62	-6.1E-01	1.1E-01
C_0067	Asp	424	HMDB0000191,HMDB0006483	134.044	9.60	-7.4E-01	-1.9E-01
C_0068	Adenine	190	HMDB0000034	136.062	6.24	-9.6E-01	2.2E-02
C_0069	Hypoxanthine	790	HMDB0000157	137.046	9.42	-6.1E-01	7.6E-01
C_0070	1-Methylnicotinamide	457	HMDB0000699	137.071	6.02	-9.2E-01	1.5E-01
C_0072	Trigonelline	5570	HMDB0000875	138.056	8.62	-8.8E-01	4.4E-01
C_0073	Tyramine	5610	HMDB0000306	138.091	6.80	-6.9E-01	-1.8E-01
C_0074	γ-Glu-Lys_divalent	65254	HMDB0029154	138.582	7.02	-7.2E-01	-3.3E-01
C_0075	Urocanic acid	736715	HMDB0000301	139.050	6.79	1.0E-01	-7.1E-02
C_0076	1*H*-Imidazole-4-propionic acid	10105257		141.066	6.64	1.2E-02	-4.4E-02
C_0077	1-Methyl-4-imidazoleacetic acid	75810	HMDB0002820	141.066	6.79	9.6E-02	-3.0E-02
C_0078	XC0029 Stachydrine	0 115244	HMDB0004827	144.101	9.44	-8.1E-01	-1.3E-01
C_0079	4-Guanidinobutyric acid	500	HMDB0003464	146.092	6.79	-5.2E-01	-8.8E-02
C_0080	γ-Butyrobetaine	134	HMDB0001161	146.118	6.63	-9.6E-01	-2.3E-01
C_0081	Acetylcholine	187	HMDB0000895	146.118	6.22	-9.7E-01	-1.9E-01
C_0082	Spermidine	1102	HMDB0001257	146.165	3.70	-5.8E-01	9.0E-02
C_0083	Lys	866	HMDB0000182,HMDB0003405	147.113	5.62	-9.8E-01	-1.6E-01
C_0084	2-Methylthiazolidine-4-carboxylic acid	160736		148.042	11.83	-6.8E-01	7.0E-01
C_0085	Isoglutamic acid	73064		148.060	7.52	5.7E-01	-5.8E-02
C_0086	*N*-Acetylserine	65249	HMDB0002931	148.060	19.78	-6.8E-01	3.5E-02
C_0087	*threo*-β-Methylaspartic acid	440064		148.060	10.22	-7.6E-02	6.0E-01
C_0088	*N*-Methylaspartic acid	22880	HMDB0002393	148.060	11.34	-9.1E-01	-3.7E-01
C_0089	Gln	738	HMDB0000641,HMDB0003423	148.079	8.88	-9.8E-01	-1.4E-01
C_0090	Glu	611	HMDB0000148,HMDB0003339	149.063	9.04	-9.5E-01	-1.0E-01
C_0091	Met	876	HMDB0000696	150.058	8.84	-9.6E-01	7.3E-02
C_0092	Triethanolamine	7618		150.112	6.74	2.5E-01	-3.5E-01
C_0093	Guanine	764	HMDB0000132	152.057	6.83	-4.4E-01	-2.8E-01
C_0094	γ-Glu-Arg_divalent	20719180	HMDB0029143	152.585	7.13	-6.5E-01	-3.2E-01
C_0095	Xanthine	1188	HMDB0000292	153.041	16.64	-5.9E-01	7.7E-01
C_0096	N^1-Methyl-4-pyridone-5-carboxamide	440810	HMDB0004194	153.066	15.59	-9.3E-01	8.4E-02
C_0097	Octopamine Dopamine	440266 681	HMDB0004825 HMDB0000073	154.087	7.11	-9.5E-01	2.6E-01
C_0098	His	773	HMDB0000177	156.077	5.98	-9.9E-01	-3.0E-02
C_0099	Imidazolelactic acid	793		157.061	7.22	-3.8E-01	4.9E-01
C_0100	XC0145 Ala-Ala	15331 5460362	HMDB0003459	161.091	7.57	-1.2E-01	-4.0E-01
C_0101	N^6-Methyllysine	164795	HMDB0002038	161.128	5.80	-9.4E-01	-2.4E-01
C_0102	*O*-Acetylhomoserine 2-Aminoadipic acid	439389 92136	HMDB0000510	162.076	9.04	-6.4E-01	-3.3E-01
C_0103	5-Hydroxylysine	3032849	HMDB0000450	163.107	5.84	-5.9E-01	-7.6E-01
C_0104	Carnitine	85	HMDB0000062	163.116	6.95	-9.6E-01	-2.2E-01
C_0105	Lumazine	10250		165.041	18.77	2.1E-01	4.4E-01
C_0106	Methionine sulfoxide	158980	HMDB0002005	166.053	9.84	-3.6E-01	-8.5E-01
C_0107	7-Methylguanine	11361	HMDB0000897	166.072	6.75	-8.7E-01	3.4E-01
C_0108	Normetanephrine_-H_2O	688100	HMDB0000819	166.086	7.48	-4.7E-01	7.6E-01
C_0109	Phe	994	HMDB0000159	166.086	9.12	-9.3E-01	3.5E-01
C_0110	Taurocyamine	68340	HMDB0003584	168.042	18.85	6.9E-01	-3.1E-01

C_0111	Pyridoxal	1050	HMDB0001545	168.065	7.18	-2.0E-01	-1.4E-01
C_0112	4-Hydroxyphenylglycine 3-Methoxyanthranilic acid	92143 255720		168.065	9.97	1.5E-01	6.7E-02
C_0113	Tyr-Arg_divalent	123804		169.594	6.29	1.1E-01	-4.6E-01
C_0114	Noradrenaline 6-Hydroxydopamine	439260 4624	HMDB0000216 HMDB0001537	170.082	7.38	-9.3E-01	3.3E-01
C_0115	1-Methylhistidine 3-Methylhistidine	92105 64969	HMDB0000001 HMDB0000479	170.092	6.13	-7.4E-01	-5.4E-01
C_0116	XC0040			174.087	9.93	-8.9E-01	4.0E-01
C_0117	*N*-Acetylornithine	439232	HMDB0003357	175.108	7.80	9.0E-02	-3.5E-01
C_0118	N^5-Ethylglutamine	439378		175.109	9.07	-9.0E-01	-1.0E-01
C_0119	Arg	6322	HMDB0000517,HMDB0003416	175.119	5.82	-9.5E-01	6.4E-02
C_0120	Guanidinosuccinic acid	439918	HMDB0003157	176.067	8.32	-8.3E-01	2.3E-02
C_0121	Citrulline	9750	HMDB0000904	176.103	9.12	-9.1E-01	-2.7E-01
C_0122	Serotonin	5202	HMDB0000259	177.103	7.17	-7.5E-01	-3.3E-01
C_0123	Alliin	87310		178.053	12.55	1.7E-01	-1.7E-01
C_0124	Gluconolactone	7027	HMDB0000150	179.055	19.61	-9.0E-01	-4.8E-02
C_0125	Glucosamine	439213	HMDB0001514	180.089	7.58	-6.3E-01	-3.5E-01
C_0126	Tyr	1153	HMDB0000158	182.081	9.36	-9.7E-01	1.4E-01
C_0127	Phosphorylcholine	1014	HMDB0001565	184.074	17.28	-9.7E-01	2.2E-01
C_0128	Adrenaline	5816	HMDB0000068	184.097	7.49	-2.8E-01	9.1E-01
C_0129	N^1-Acetylspermidine	496	HMDB0001276	188.176	5.15	-9.8E-01	-2.0E-02
C_0130	*N*-Acetyllysine	92907	HMDB0000446	189.123	8.01	-4.9E-01	-2.4E-01
C_0131	Gly-Leu			189.123	7.90	1.2E-01	-2.6E-01
C_0132	N^6-Acetyllysine	92832	HMDB0000206	189.123	9.42	-5.8E-01	-1.2E-01
C_0133	N_G,Methylarginine	132862		189.134	6.09	-8.9E-01	4.1E-01
C_0134	N^6,N^6,N^6-Trimethyllysine	440120	HMDB0001325	189.160	5.86	-8.9E-01	-2.5E-01
C_0135	Homocitrulline	65072	HMDB0000679	190.118	9.20	-8.1E-01	-2.8E-01
C_0136	Gly-Asp	97363		191.067	8.12	-9.9E-01	-3.1E-02
C_0137	4-Aminohippuric acid	2148	HMDB0001867	195.078	8.38	-2.8E-01	9.1E-01
C_0138	Tyrosine methyl ester	70652		196.097	7.50	1.7E-01	-1.1E-01
C_0139	*N*-Acetylhistidine	75619		198.087	8.04	-6.2E-01	-2.9E-02
C_0140	ADMA	123831	HMDB0001539	203.150	6.26	-9.5E-01	1.3E-01
C_0141	SDMA	169148	HMDB0003334	203.150	6.36	-9.1E-01	-3.6E-02
C_0142	Spermine	1103	HMDB0001256	203.223	3.65	-2.4E-01	8.1E-02
C_0143	*O*-Acetylcarnitine	439756	HMDB0000201	204.122	7.40	-9.9E-01	-8.2E-02
C_0144	γ-Glu-Gly	165527	HMDB0011667	205.082	9.96	-7.8E-01	5.8E-01
C_0145	Trp	1148	HMDB0000929	205.097	9.07	-8.6E-01	-9.0E-02
C_0146	Carboxymethyllysine	123800		205.119	7.58	-9.7E-01	-1.0E-01
C_0147	Lipoamide	863	HMDB0000962	206.069	19.18	-2.8E-01	9.1E-01
C_0148	Kynurenine	846	HMDB0000684	209.092	8.27	-9.6E-01	-2.4E-01
C_0149	Propionylcarnitine XC0061	188824 0	HMDB0000824	218.138	7.63	-8.3E-01	-8.1E-02
C_0150	β-Ala-Lys	440638		218.150	5.53	-1.8E-01	-3.6E-01
C_0151	γ-Glu-Ala	440103	HMDB0006248	219.098	10.18	-7.4E-01	2.5E-01
C_0152	XC0065			221.091	10.84	-9.7E-01	-1.8E-01
C_0153	*N*-Acetylglucosylamine	439454	HMDB0001104	221.112	8.12	-5.4E-01	2.7E-01
C_0154	*N*-Acetylgalactosamine *N*-Acetylglucosamine *N*-Acetylmannosamine	35717 439174 439281	HMDB0000853 HMDB0000215 HMDB0001129	222.097	18.87	-6.4E-01	-1.5E-01
C_0155	Cystathionine	834	HMDB0000099	223.075	8.16	-9.5E-01	-2.7E-01
C_0156	Neostigmine	4456		223.145	7.29	7.5E-02	-2.5E-01
C_0157	3-Hydroxykynurenine	11811	HMDB0011631	225.085	8.16	-9.1E-01	-3.7E-01
C_0158	Carnosine	439224	HMDB0000033	227.114	5.51	-9.4E-01	-2.9E-01
C_0159	2'-Deoxycytidine	13711	HMDB0000014	228.098	7.73	-4.5E-01	-1.9E-01
C_0160	Butyrylcarnitine	439829	HMDB0002013	232.154	7.83	-9.0E-01	-3.0E-01
C_0161	Isobutyrylcarnitine	168379	HMDB0000736	232.154	7.77	-4.0E-01	-1.6E-01
C_0162	γ-Glu-Ser	22844748	HMDB0029158	235.093	10.42	-9.2E-01	2.5E-01
C_0163	Thr-Asp	3280446		235.093	8.73	-9.2E-01	-3.9E-02
C_0164	Ser-Glu			235.093	8.54	-9.8E-01	-1.0E-01
C_0165	*N*'-Formylkynurenine	910	HMDB0001200	237.087	10.05	-9.1E-01	-3.7E-01
C_0166	Cystine	595	HMDB0000192	241.032	9.09	4.2E-01	-2.8E-01
C_0167	Homocarnosine	10243361	HMDB0000745	241.130	5.57	-9.6E-01	-2.2E-01
C_0168	Thymidine	5789	HMDB0000273	243.098	18.85	-2.1E-01	-2.6E-01
C_0169	Cytidine	6175	HMDB0000089	244.093	7.93	-8.3E-01	5.3E-01
C_0170	Uridine	6029	HMDB0000296	245.077	18.89	-7.8E-01	5.8E-01
C_0171	N^1-Acetylspermine	916	HMDB0001186	245.233	4.51	-9.1E-01	-3.7E-01
C_0172	Isovalerylcarnitine	6426851	HMDB0000688	246.170	8.00	-7.0E-01	9.0E-02
C_0173	γ-Glu-Val	7015683	HMDB0011172	247.129	10.51	-9.7E-01	-7.9E-02
C_0174	Malonylcarnitine	22833583	HMDB0002095	248.112	8.37	-9.2E-01	-1.8E-01
C_0175	Pyridoxamine 5'-phosphate	1053	HMDB0001555	249.063	8.61	-9.5E-01	4.8E-02

C_0176	γ-Glu-Thr	53861142	HMDB0029159	249.108	10.52	-9.5E-01	-2.3E-01
C_0177	γ-Glu-Cys	123938	HMDB0001049	251.069	10.60	-7.9E-01	1.5E-01
C_0178	2'-Deoxyinosine	135398593	HMDB0000071	253.094	15.41	1.8E-01	-8.6E-02
C_0179	XC0089			255.098	7.80	-9.9E-01	-3.3E-02
C_0180	XC0154	3182		255.107	18.93	5.1E-01	-4.8E-01
C_0181	Glycerophosphocholine	439285	HMDB0000086	258.108	18.43	-9.9E-01	-3.0E-02
C_0182	γ-Glu-Ile γ-Glu-Leu	22885096 151023	HMDB0011170 HMDB0011171	261.143	10.68	-9.6E-01	2.5E-03
C_0183	γ-Glu-Asn	131801686	HMDB0029144	262.103	10.56	-8.9E-01	4.1E-01
C_0184	γ-Glu-Ornitine	189156	HMDB0002248	262.140	6.95	-4.7E-01	-3.4E-02
C_0185	γ-Glu-Asp	161197	HMDB0030419	263.088	10.76	-9.1E-01	-6.9E-02
C_0186	Thiamine	1130	HMDB0000235	265.111	5.35	-9.5E-01	2.7E-01
C_0187	Adenosine	60961	HMDB0000050	268.103	8.11	-9.3E-01	-3.3E-01
C_0188	Inosine	6021	HMDB0000195	269.088	16.60	-8.7E-01	4.4E-01
C_0189	γ-Glu-Gln	150914	HMDB0011738	276.119	10.77	-9.7E-01	1.2E-01
C_0190	Glu-Glu	439500		277.104	8.96	-6.6E-01	-4.7E-02
C_0191	γ-Glu-Glu	92865	HMDB0011737	277.103	10.86	-6.9E-01	6.1E-01
C_0192	Saccharopine	160556	HMDB0000279	277.139	8.84	-9.4E-01	-2.9E-01
C_0193	γ-Glu-Met	7009567	HMDB0034367	279.101	10.73	-9.1E-01	-3.7E-01
C_0194	1-Methyladenosine	27476	HMDB0003331	282.117	8.17	-9.7E-01	1.7E-01
C_0195	Guanosine	6802	HMDB0000133	284.099	10.51	-9.6E-01	2.2E-01
C_0196	γ-Glu-His	7017195	HMDB0029151	285.120	7.13	-9.8E-01	1.2E-01
C_0197	Octanoylcarnitine	11953814	HMDB0000791	288.217	8.52	-9.5E-01	-1.6E-01
C_0198	Ophthalmic acid	7018721	HMDB0006765	290.134	10.95	-1.5E-01	-1.6E-01
C_0199	Argininosuccinic acid	16950	HMDB0000052	291.130	7.75	-7.8E-01	3.7E-01
C_0200	γ-Glu-Phe	111299	HMDB0000594	295.130	10.78	-8.4E-01	-7.7E-02
C_0201	5'-Deoxy-5'-methylthioadenosine	439176	HMDB0001173	298.097	8.28	-9.4E-01	-3.1E-01
C_0202	N^1-Methylguanosine	96373	HMDB0001563	298.115	9.97	-9.4E-01	2.7E-01
C_0203	Arg-Glu			304.161	6.12	-3.1E-01	1.1E-01
C_0204	Glutathione (GSSG)_divalent	65359	HMDB0003337	307.084	10.07	-9.9E-01	-1.1E-01
C_0205	Glutathione (GSH)	124886	HMDB0000125	308.092	10.97	-6.9E-01	2.6E-01
C_0206	XC0126			310.116	12.48	-6.6E-01	-4.7E-01
C_0207	Tyr-Glu			311.122	9.16	-3.2E-01	2.4E-01
C_0208	γ-Glu-Tyr	94340	HMDB0011741	311.123	10.95	-7.5E-02	5.2E-01
C_0209	*S*-Methylglutathione	115260		322.107	11.08	-9.8E-01	9.4E-02
C_0210	XC0132			325.161	7.10	-9.6E-01	-1.6E-01
C_0211	NMN	14180	HMDB0000229	335.065	17.41	-8.9E-01	-3.9E-01
C_0212	Lauroylcarnitine	168381	HMDB0002250	344.280	9.14	-9.2E-01	-2.3E-01
C_0213	Thiamine phosphate	1131	HMDB0002666	345.078	8.78	-9.8E-01	1.5E-02
C_0214	XC0137			350.099	11.19	-9.4E-01	-1.5E-01
C_0215	Decarboxylated *S*-Adenosylmethionine	439415	HMDB0000988	355.157	4.58	1.7E-01	-1.7E-01
C_0216	Riboflavin	493570	HMDB0000244	377.147	18.76	-9.2E-01	2.6E-01
C_0217	*S*-Lactoylglutathione	440018	HMDB0001066	380.114	11.45	-9.1E-01	-3.7E-01
C_0218	*S*-Adenosylhomocysteine	439155	HMDB0000939	385.129	7.17	-7.0E-01	7.0E-01
C_0219	*S*-Adenosylmethionine	34755	HMDB0001185	399.144	5.83	-9.7E-01	-2.0E-01
C_0220	Cysteine glutathione disulfide	10455148	HMDB0000656	427.098	9.63	-3.9E-01	2.3E-01

MT, migration time; PC, principal component; RT, retention time

3.

Supplementary Table S3. Results of comparative analysis

ID	Metabolite	PubChem CID	HMDB ID	Concentration (nmol/g)												Comparative Analysis	
				treatment					Control					treatment		Control		treatment vs Control	
				wheyd	wheyd	wheyd	wheyd	wheyd	caseind	caseind	caseind	caseind	caseind	Mean	S.D.	Mean	S.D.	Ratio[¶]	p-value[‖]
A_0009	2-Hydroxybutyric acid	440864	HMDB0000008	0.6	N.D.	1.8	1.1	N.D.	1.2	2.8	N.D.	2.1	1.6	1.2	0.6	1.9	0.7	0.6	0.2
A_0035	2-Oxoglutaric acid	51	HMDB0000208	N.D.	N.D.	4.5	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	4.5	N.A.	N.A.	N.A.	1<	N.A.
A_0013	3-Oxoisovaleric acid	49	HMDB0000019	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
A_0070	2-Phosphoglyceric acid	439758	HMDB0003391	4.2	N.D.	2.5	2.6	1.0	N.D.	2.6	1.9	0.8	N.D.	2.6	1.3	1.8	0.8	1.5	0.6
A_0008	3-Hydroxybutyric acid	441	HMDB0000011;HMDB0000357;HMDB0003340	37	15	51	31	26	23	49	44	95	12	32	14	44	32	0.7	0.5
A_0071	3-Phosphoglyceric acid	439183	HMDB0000807	29	4.5	17	16	6.0	14	16	13	5.4	7.5	14	9.8	11	4.5	1.3	0.5
A_0112	6-Phosphogluconic acid	91483	HMDB0001316	8.7	1.5	4.1	2.4	1.3	N.D.	N.D.	0.4	1.4	2.6	3.6	3.1	1.5	1.1	2.4	0.2
A_0144	Acetyl CoA_divalent	444493		N.D.	N.D.	0.2	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.2	N.A.	N.A.	N.A.	1<	N.A.
C_0088	Adenine	190	HMDB0000034	0.3	0.08	0.7	0.2	0.2	0.06	0.10	0.09	0.07	0.08	0.3	0.2	0.08	0.02	4.1	0.1
C_0187	Adenosine	60961	HMDB0000050	6.4	0.5	109	3.6	3.5	1.3	2.2	2.0	1.2	0.7	25	47	1.5	0.6	16.6	0.3
A_0168	ADP	6022	HMDB0001341	12	2.1	152	5.9	3.8	4.9	5.4	3.1	3.6	2.8	35	66	3.9	1.1	9.0	0.3
C_0011	Ala	602	HMDB0000161;HMDB0000310	110	75	133	98	81	89	110	92	87	120	99	23	98	14	1.0	1.0
A_0130	AMP	6083	HMDB0000045	32	26	170	34	67	70	110	74	62	47	66	60	73	23	0.9	0.8
C_0071	Anthranilic acid	227	HMDB0011723	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
C_0119	Arg	6322	HMDB0000517;HMDB0003416	23	6.1	34	9.6	15	12	13	11	13	13	17	11	13	0.8	1.4	0.4
C_0063	Asn	236	HMDB0000168;HMDB0029783	16	5.8	25	16	9.2	8.4	11	9.6	9.7	10	13	7.3	9.7	0.8	1.4	0.3
C_0067	Asp	424	HMDB0000191;HMDB0004493	44	26	61	31	24	27	55	32	44	35	37	16	38	11	1.0	0.9
A_0159	ATP	5957	HMDB0000538	6.6	0.3	189	1.5	0.5	0.7	0.8	0.3	0.4	0.2	39	83	0.5	0.3	82.2	0.4
C_0036	Betaine	247	HMDB0000043	48	9.6	117	21	9.9	13	12	10	7.9	12	41	45	11	2.0	3.7	0.2
C_0040	Betaine aldehyde_+H2O	249		2.0	0.4	1.2	1.5	0.7	0.6	0.2	0.4	0.5	1.5	1.2	0.6	0.7	0.5	1.7	0.2
A_0127	cAMP	6076	HMDB0000058	N.D.	N.D.	1.0	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	1.0	N.A.	N.A.	N.A.	1<	N.A.
C_0158	Carnosine	439234	HMDB0000033	2.8	0.3	28	0.6	0.2	0.5	0.3	0.3	0.05	0.3	6.4	12	0.3	0.2	21.6	0.3
A_0142	CDP	6130	HMDB0001546	N.D.	N.D.	0.9	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.9	N.A.	N.A.	N.A.	1<	N.A.
A_0129	cGMP	26218	HMDB0001314	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
C_0034	Choline	305	HMDB0000097	123	27	186	63	55	45	42	30	40	38	93	67	39	5.5	2.4	0.1
A_0062	cis-Aconitic acid	643757	HMDB0000072	2.1	0.8	4.3	1.2	0.7	1.7	1.6	1.7	1.7	1.3	1.8	1.5	1.6	0.2	1.2	0.7
A_0081	Citric acid	311	HMDB0000094	48	24	113	38	18	34	37	30	35	34	48	38	32	5.2	1.5	0.6
C_0121	Citrulline	9750	HMDB0000904	8.3	4.8	17	8.7	6.7	9.4	8.7	6.9	6.8	6.7	9.1	4.8	7.7	1.3	1.2	0.6
A_0124	CMP	6131	HMDB0000095	2.9	2.5	9.8	4.5	3.3	4.6	5.5	3.9	4.5	4.5	4.6	3.0	4.6	0.8	1.0	1.0
A_0139	CoA_divalent	87642		0.3	1.7	0.3	0.5	0.4	N.D.	N.D.	N.D.	N.D.	N.D.	0.6	0.6	N.A.	N.A.	1<	N.A.
C_0064	Creatine	586	HMDB0000064	262	62	875	98	71	131	84	60	84	65	273	346	85	28	3.2	0.3
C_0031	Creatinine	588	HMDB0000562	2.7	0.8	5.1	1.1	1.0	1.9	2.0	2.6	4.1	0.9	2.1	1.8	2.3	1.2	0.9	0.9
A_0155	CTP	6178	HMDB0000563	N.D.	N.D.	1.8	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	1.8	N.A.	N.A.	N.A.	1<	N.A.
C_0043	Cys	594	HMDB0000574;HMDB0030417	0.11	N.D.	0.09	N.D.	N.D.	N.D.	0.09	N.D.	0.07	N.D.	0.10	0.02	0.08	0.011	1.2	0.4
C_0189	Cytidine	6175	HMDB0000089	8.1	1.9	7.1	3.9	2.2	3.0	2.0	1.4	2.0	2.0	4.6	2.9	2.1	0.5	2.2	0.1
C_0038	Cytosine	597	HMDB0000630	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
A_0158	dATP	15993	HMDB0001532	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
A_0153	dCTP	65081	HMDB0000998	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
A_0056	Dihydroxyacetone phosphate	668	HMDB0001473	13	1.9	25	4.6	2.0	2.4	1.4	1.2	3.8	4.0	9.3	9.9	2.5	1.3	3.7	0.2
A_0141	dTDP	16218276	HMDB0001274	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
A_0122	dTMP	6700	HMDB0001227	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
A_0154	dTTP	64968	HMDB0001342	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
A_0067	Erythrose 4-phosphate	122137	HMDB0001321	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
A_0128	Fructose 1,6-diphosphate	172153	HMDB0001058	29	0.8	54	6.4	1.1	6.3	1.5	1.4	4.4	6.8	18	23	3.7	2.2	5.0	0.2
A_0107	Fructose 6-phosphate	826	HMDB0001076	12	0.4	11	2.1	1.4	1.1	N.D.	0.5	1.9	2.4	5.5	5.8	1.5	0.8	3.8	0.2
A_0012	Fumaric acid	444972	HMDB0000134	13	N.D.	18	8.1	4.0	5.9	8.4	N.D.	6.3	5.8	11	6.0	6.6	1.2	1.7	0.2
C_0031	GABA	119	HMDB0000112	2.1	1.6	19	2.2	2.2	2.0	2.5	1.0	2.6	1.6	5.3	7.4	1.9	0.7	2.8	0.4
A_0150	GDP	8977	HMDB0001201	2.1	0.7	6.2	1.8	2.0	1.1	2.1	1.2	1.7	1.2	2.6	2.1	1.5	0.4	1.8	0.3
C_0089	Gln	738	HMDB0000641;HMDB0003423	240	127	553	180	148	135	155	133	139	164	230	175	139	11	1.8	0.2
C_0093	Glu	611	HMDB0000148;HMDB0003339	258	119	498	193	139	108	264	112	169	111	241	154	152	67	1.6	0.3
A_0089	Gluconic acid	10969	HMDB0000625	5.3	3.7	7.8	4.7	2.5	2.6	4.0	3.1	3.9	3.2	4.8	1.9	3.4	0.8	1.4	0.2
A_0106	Glucose 1-phosphate	65533	HMDB0001586	7.7	0.7	8.8	3.4	1.2	1.8	1.2	1.5	1.4	2.1	4.1	3.7	1.6	0.4	2.6	0.2
A_0110	Glucose 6-phosphate	5958	HMDB0001401	57	1.8	33	8.8	5.2	0.9	0.9	0.8	0.5	11	21	24	4.1	4.7	5.2	0.2
C_0205	Glutathione (GSH)	124886	HMDB0061705	16	9.1	25	22	13	0.3	0.2	3.1	1.8	7.1	17	9.4	2.4	2.8	7.1	0.0 **
C_0204	Glutathione (GSSG)_divalent	65359		76	13	167	43	34	32	56	32	40	35	66	61	39	10	1.7	0.4
C_0095	Gly	750	HMDB0000123	289	65	418	140	94	88	90	79	65	75	201	149	78	11	2.6	0.1
A_0057	Glyceraldehyde 3-phosphate	729	HMDB0001112	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
A_0059	Glycerol 3-phosphate	439162	HMDB0000126	210	36	257	114	75	45	70	53	62	61	138	93	58	9.7	2.4	0.1
A_0052	Glycolic acid	757	HMDB0000115	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
A_0051	Glyoxylic acid	760	HMDB0000119	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
A_0135	GMP	8964	HMDB0001397	8.2	7.5	32	13	18	19	27	19	21	18	19	10	20	4.1	0.8	0.4
A_0161	GTP	6830	HMDB0001273	1.7	N.D.	11	0.7	0.4	N.D.	0.4	N.D.	0.4	N.D.	3.4	5.0	0.4	0.03	9.0	0.3
C_0083	Guanine	764	HMDB0000132	N.D.	0.02	0.3	0.2	N.D.	0.4	N.D.	N.D.	N.D.	N.D.	0.2	0.15	0.4	N.A.	0.4	N.A.
C_0185	Guanosine	6802	HMDB0000133	11	1.4	17	5.3	2.7	2.2	1.0	1.2	1.5	1.5	7.5	6.5	1.5	0.5	5.1	0.1
C_0088	His	773	HMDB0000177	17	6.1	36	11	10	7.9	12	8.7	8.5	9.2	15	9.3	9.2	1.4	1.6	0.2
C_0039	Homoserine	12647	HMDB0062715	0.3	N.D.	0.5	0.2	N.D.	0.11	N.D.	0.15	0.2	0.15	0.3	0.2	0.14	0.02	2.4	0.2
C_0057	Hydroxyproline	5810	HMDB0000725	6.8	3.5	16	4.9	3.3	2.4	4.4	3.0	3.3	3.5	6.9	5.3	3.3	0.7	2.1	0.2
C_0069	Hypoxanthine	790	HMDB0000157	92	30	52	31	24	16	9.3	10	19	23	46	28	16	6.0	2.9	0.1
C_0081	Ile	791	HMDB0000172	21	7.5	26	13	11	7.7	9.8	7.9	8.8	7.1	16	7.6	8.3	1.1	1.9	0.1
A_0132	IMP	8582	HMDB0000175	33	8.3	63	19	27	14	28	17	26	44	30	21	26	12	1.2	0.7
C_0188	Inosine	6021	HMDB0000195	204	56	212	83	58	23	17	19	26	29	121	81	23	5.4	5.2	0.1
A_0060	Isocitric acid	1198	HMDB0000193	N.D.	N.D.	1.2	N.D.	N.D.	N.D.	1.1	N.D.	1.4	0.9	1.2	N.A.	1.2	0.2	1.1	N.A.
A_0006	Lactic acid	612	HMDB0000190;HMDB0003311	866	452	2,084	594	396	566	635	484	537	496	661	620	544	60	1.8	0.3
C_0060	Leu	857	HMDB0000687	39	14	47	24	22	15	20	16	16	16	28	13	17	2.8	1.7	0.1
C_0083	Lys	866	HMDB0000182;HMDB0003405	40	16	66	26	25	24	33	28	21	28	38	28	27	4.4	1.5	0.4
A_0027	Malic acid	525	HMDB0000156;HMDB0000745	71	31	74	47	24	36	49	27	47	40	49	23	40	8.8	1.2	0.4
A_0147	Malonyl CoA_divalent	6034595		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
C_0061	Met	876	HMDB0000696	6.8	4.1	13	6.2	6.6	5.0	6.8	5.7	6.8	6.8	8.1	3.7	6.2	0.9	1.3	0.3
C_0020	Al_N-Dimethylglycine	673	HMDB0000092	0.7	0.4	1.0	0.8	0.6	0.7	0.8	0.5	0.5	0.6	0.7	0.2	0.6	0.15	1.1	0.6
A_0169	NAD^+	5892	HMDB0000902	4.2	0.3	60	1.9	1.0	2.5	2.3	2.2	0.2	0.4	13	26	1.5	1.1	8.8	0.4
A_0167	NADP$^+$	5886	HMDB0000217	6.6	N.D.	18	2.4	0.6	0.7	1.3	1.0	0.6	0.4	7.5	7.9	0.8	0.3	9.7	0.2
C_0065	Ornithine	389	HMDB0000214;HMDB0003374	11	5.4	8.3	10	4.8	5.0	7.9	7.1	3.5	6.9	8.0	2.8	6.0	2.0	1.3	0.2
C_0109	Pi	604	HMDB0000106	21	6.6	23	10	11	7.3	10	8.0	6.4	9.2	14	7.6	8.8	1.5	1.6	0.2
A_0063	Phosphoenolpyruvic acid	1005	HMDB0000263	9.7	1.6	5.8	6.1	2.6	4.7	5.8	4.1	3.8	2.7	5.2	3.2	3.8	1.8	1.3	0.4
C_0033	Pro	614	HMDB0000162;HMDB0003411	24	12	38	16	14	17	21	13	17	15	21	11	16	2.8	1.3	0.4
A_0139	PRPP	7339	HMDB0000295	N.D.	N.D.	0.8	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.8	N.A.	N.A.	N.A.	1<	N.A.
C_0009	Putrescine	1045	HMDB0001414	4.9	0.8	5.7	1.1	0.7	1.1	2.1	1.3	1.4	1.2	2.6	2.4	1.6	0.4	1.9	0.3
A_0064	Pyruvic acid	1060	HMDB0000243	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
A_0100	Ribose 5-phosphate	439167	HMDB0001548	14	7.9	4.3	8.7	5.2	3.2	2.9	2.4	2.8	2.8	7.6	3.7	2.8	0.3	2.7	0.0 *
A_0099	Ribulose 5-phosphate	439184	HMDB0001518	91	49	58	46	35	15	17	18	16	18	58	21	17	1.5	3.3	0.0 *
C_0219	S-Adenosylmethionine	34756	HMDB0001185	1.3	0.4	4.7	0.9	0.5	0.5	0.9	0.4	0.8	0.4	1.6	1.8	0.5	0.2	2.9	0.3
C_0012	Sarcosine	1088	HMDB0062711	15	0.8	143	1.3	N.D.	0.3	N.D.	0.4	N.D.	0.5	40	68	0.4	0.11	94.0	0.3
A_0115	Sedoheptulose 7-phosphate	165937	HMDB0001068	13	2.0	7.8	8.1	7.1	2.6	4.4	3.4	3.7	4.4	7.9	4.1	3.7	0.8	2.1	0.1
C_0035	Ser	617	HMDB0000187;HMDB0003406	80	32	112	58	48	33	40	37	36	43	63	33	38	3.6	1.7	0.2
C_0082	Spermidine	1102	HMDB0001257	10	11	14	9.0	2.9	3.7	5.5	5.8	2.8	10	9.4	4.0	5.5	3.1	1.7	0.1
C_0142	Spermine	1103	HMDB0001256	4.7	8.8	6.3	5.5	2.4	1.3	1.4	3.3	0.8	6.3	5.6	2.4	2.6	2.2	2.1	0.1
A_0076	Succinic acid	1110	HMDB0000254	25	15	72	29	24	20	37	22	21	14	33	22	23	6.5	1.4	0.4
C_0038	Thr	6288	HMDB0000167	46	19	91	33	28	23	27	28	26	26	43	28	26	2.1	1.7	0.2
C_0188	Thymidine	5789	HMDB0000273	0.3	0.3	0.5	0.4	0.3	0.8	0.5	0.3	0.2	0.3	0.4	0.18	0.4	0.2	0.8	0.6
C_0060	Thymine	1135	HMDB0062662	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
C_0145	Trp	1148	HMDB0000929	5.8	1.5	9.1	4.7	4.6	4.0	5.5	4.6	4.7	4.5	5.0	2.9	4.7	0.8	1.1	0.8
C_0126	Tyr	1153	HMDB0000158	17	5.9	22	12	10	8.5	10	9.5	9.5	9.6	13	6.2	9.5	0.7	1.4	0.2
C_0073	Tyramine	5610	HMDB0000306	0.06	0.06	0.11	0.08	0.08	0.05	0.06	0.03	0.05	0.06	0.08	0.02	0.05	0.012	1.5	0.1
A_0143	UDP	6031	HMDB0000295	0.4	N.D.	3.8	0.3	0.2	0.2	0.4	N.D.	0.2	0.2	1.1	1.7	0.2	0.07	4.8	0.4
A_0125	UMP	6030	HMDB0000296	7.8	7.8	31	10	11	16	23	17	17	16	14	9.6	18	2.7	0.8	0.4
C_0030	Uracil	1174	HMDB0000300	11	5.4	7.8	8.1	8.3	5.2	5.7	4.7	5.9	6.1	8.1	2.0	5.5	0.8	1.5	0.0 *
C_0170	Uridine	6029	HMDB0000296	34	14	28	22	15	11	11	9.3	11	10	23	8.7	10	0.8	2.2	0.0 *
A_0156	UTP	6133	HMDB0000285	0.2	N.D.	4.4	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	2.3	3.0	N.A.	N.A.	1<	N.A.
C_0035	Val	1182	HMDB0000883	36	16	53	29	26	20	24	20	16	19	33	14	20	2.3	1.6	0.1
C_0010	β-Ala	239	HMDB0000056	8.0	3.9	27	5.1	4.9	2.4	4.6	3.0	3.1	3.5	9.7	9.6	3.3	0.8	3.0	0.2

N.A., Not Available; N.D., Not Detected
¶ The ratio of the detected mean values between the two groups.
‖ Welch's t-test (*p<0.05, **p<0.01, ***p<0.001)

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