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Whey Protein Intake Modulates Lipid Metabolism by Transcriptionally Affecting PPARs and SREBP1c and Their Downstream Enzymes in Mice

Hajime Sasaki

Department of Nutrition and Life Sciences, Kanagawa-Institute of Technology, Atsugi, Japan
Email: sasaki@cco.kanagawa-it.ac.jp

Abstract

Background: The effects of whey protein intake on the transcriptional expression of genes related to lipid metabolism in mice were investigated herein. **Methods:** For 4 weeks, mice were fed AIN-93G composed of either casein or whey protein as the protein source. Then the gastrocnemius muscle, liver, and epididymal adipose tissue were excised. Expression levels of the transcription factors, PPARα, PPARγ, and SREBP1c, and those of the enzymes modulated by these factors, HSL, LPL, ACCα, and FAS, were measured by real-time PCR. The effects of whey protein were compared to those of the case in control. **Results:** The mRNA expression of PPARα was enhanced in the gastrocnemius muscle, while that of PPARγ was increased in the liver and epididymal adipose tissue. The expression of HSL and LPL was increased in the epididymal adipose tissue and gastrocnemius muscle, respectively. The mRNA expression of SREBP1c was suppressed in all of the three tissues. The expression of ACCα was suppressed in the gastrocnemius muscle and liver, while that of FAS was suppressed in all of the three tissues. **Conclusions:** These results indicate that whey protein intakes transcriptionally modulate PPARs and SREBP1c directing lipid metabolism toward the enhancement of triglyceride breakdown and suppression of fatty acid synthesis.

1. Introduction

Whey protein in cow's milk is a nutritionally beneficial source of protein, which

has well-balanced amino acid ratios. It is also known that whey protein efficiently enhances protein synthesis in skeletal muscles [1] [2] [3] by activating a pathway regulated by mechanistic target of rapamycin (mTOR) [4] [5], and its ability to enhance protein synthesis is more potent than casein or soy protein [6]. Additionally, whey protein exhibits various physiological functions including suppression of inflammation.

The anti-inflammatory function of whey protein was shown in animal inflammatory models such as the galactosamine-induced hepatitis model [7], which reproduces chemical-induced hepatitis, and the ConA-induced hepatitis model [8], caused by immunological reactions. The anti-inflammatory effect of whey protein was shown in human-beings as well. In chronical obstructive pulmonary disease (COPD) patients, a liquid diet comprising whey protein peptides as a protein source suppressed inflammatory reactions [9]. It also reduced the risk of bacteremia post hepatic transplantation surgery [10].

These data suggest that whey protein might have therapeutic applications in clinical problems where protein deficiency and inflammatory reactions cause symptoms, for instance, sarcopenia, which is characterized by age-related loss of muscle mass and quality. Because loss of muscle protein is enhanced by declined protein intake and chronic low-grade inflammation associated with the aged [11], the prerequisites for clinical therapy of sarcopenia should be protein supplementation and suppression of inflammation. Whey protein has both these properties making it a potential therapeutic candidate for sarcopenia.

On the other hand, sarcopenia is often associated with type 2 diabetes [12] [13], as loss of muscle mass and chronic inflammation enhance the progression of insulin resistance leading to the onset of diabetes. A stronger association of type 2 diabetes with sarcopenia is observed in patients with sarcopenia obesity, a comorbidity of sarcopenia and obesity [14]. Accumulated mass of adipose tissues in sarcopenia obesity is thought to be a cause of aggravation of insulin resistance, as it causes adipose tissue-related inflammation and systemic dyslipidemia exacerbating insulin resistance [15].

For the therapeutic application of whey protein for sarcopenia and sarcopenia obesity, it is important to know how whey protein affects lipid metabolism. This study describes how whey protein intake affects expression patterns of genes related to lipid metabolism in mice. A pathway for the regulation of triglyceride degradation by the transcription factors, peroxisome proliferator-activated receptors (PPARs), and their target enzymes, hormone-sensitive lipase (HSL) and lipoprotein lipase (LPL), and a pathway for the regulation of fatty acid synthesis, the transcription factor, sterol regulatory element-binding transcription factor 1 (SREBP1c), and its target enzymes, acetyl-CoA carboxylase alpha (ACCa) and fatty acid synthase (FAS), were analyzed.

2. Materials and Methods

2.1. Animal Experiments

Animal experiments were conducted in accordance with the Guidelines for

Proper Conduct of Animal Experiments (The Science Council of Japan) after approval by the Animal Ethical Care Committee of Kanagawa Institute of Technology.

Male, 6-week-old, C57BL mice were purchased from Charles River Laboratories (Japan) and were separated into two groups (n = 5). They were fed AIN-93G (Oriental Yeast, Japan), comprising casein as the protein source for 5 days to acclimate to environment and diet. For the next 4 weeks, one group was fed AIN-93G composed of casein and the other AIN-93G composed of whey protein (Arla Foods Ingredients, Japan) as the protein source. Mice were maintained at constant room temperature of 20°C - 22°C with free access to water and diet under a 12:12 h light-dark cycle with lights on at 7:00 AM.

The gastrocnemius muscle, liver, and epididymal adipose tissue of these mice were excised, immersed, and stored in RNALater (Thermo Fischer Scientific, USA) at −20°C.

2.2. Real-Time PCR

The protocol provided with ReliaPrep RNA Miniprep Systems (Promega, USA) was used to separate total RNA from tissues. Reverse transcription of RNA to cDNA was performed using ReverTra Ace qPCR RT Master Mix (TOYOBO, Japan) on ABI Geneamp 9700 PCR-Thermal Cycler (Applied Biosystems, USA).

Real-time PCR was performed using KOD-Plis-Ver.2 (TOYOBO, Japan) on ABI Applied Biosystems 7300 Real-Time PCR System (Applied Biosystems, USA). Primers used to detect and quantify gene expression were synthesized using TKARA's (Japan) database. Sequences are shown in Table 1.

Table 1. List of DNA primer sets for quantitative real-time PCR.

Gene	Direction	Sequence	Start position	Size	Variant coverage
18S rRNA	Forward	TTCTGGCCAACGGTCTAGACAAC	314	127	-
	Reverse	CCAGTGGTCTTGGTGTGCTGA	440		
FAS	Forward	ATTGGCTCCACCAAATCCAAC	1491	90	-
	Reverse	CCCATGCTCCAGGGATAACAG	1580		
HSL	Forward	TCCTGGAACTAAGTGGACGCAAG	2093	93	2/2
	Reverse	CAGACACACTCCTGCGCATAGAC	2185		
LPL	Forward	AGAGGCTATAGCTGGGAGCAGAAAC	3440	137	-
	Reverse	GCAAGGGCTAACATTCCAGCA	3616		
PPARα	Forward	CTCAGGGTACCACTACGGAGTTCAC	1579	153	4/4
	Reverse	TGAATCTTGCAGCTCCGATCAC	1731		
PPARγ	Forward	GGAGCCTAAGTTTGAGTTTGCTGTG	1579	153	4/4
	Reverse	TGCAGCAGGTTGTCTTGGATG	1731		
SREBP1	Forward	CCGAGATGTGCGAACTGGA	131	83	2/4
	Reverse	GAAGTCACTGTCTTGGTTGTTGATG	213		

Gene expression levels were normalized to the expression level of the internal control gene, 18S rRNA, and expressed as the mean with the standard error of mean in figures.

2.3. Statistical Analysis

The results were statistically analyzed using a Student's t-test.

3. Results

3.1. Body Weight

Body weights of mice fed casein or whey protein were 25.57 ± 0.6 g and 25.1 ± 0.8 g (mean ± standard error), respectively. No significant difference was detected between them.

3.2. Gastrocnemius Muscle

Because triglyceride degradation is regulated by PPAR pathways, the mRNA expression levels of genes associated with these pathways were measured. And since gene expression levels of the enzymes for lipid degradation, HSL and LPL, are upregulated by the activation of PPARs, the expression levels of these enzymes were also measured. In whey protein fed mice, the mRNA expression of PPARα increased, while that of PPARγ decreased. Significant decrease in mRNA expression of HSL and significant increase in LPL were observed (Figure 1).

Fatty acid synthesis is regulated by SREBP1c pathways. Activated SREBP1c upregulates gene expression levels of ACCα and FAS, two major enzymes for the fatty acid synthesis. In whey protein fed mice, the mRNA expression of SREBP1c
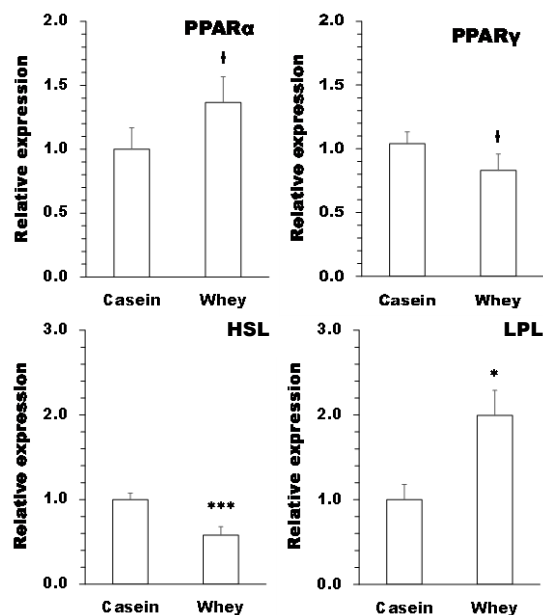
Figure 1. Effect of whey protein on mRNA levels of the enzymes related to triglyceride breakdown in the gastrocnemius muscle. The mRNA expression levels were normalized to the casein control. ɫ: P < 0.1, *: P < 0.05, ***: P < 0.001.

was significantly decreased. Consequently, the mRNA expression levels of ACCα and FAS were also significantly decreased (Figure 2).

### 3.3. Liver

In whey protein fed mice, the mRNA expression of PPARα was significantly decreased, while PPARγ was significantly increased. Whey protein significantly increased the mRNA expression of HSL, while it did not significantly change that of LPL (Figure 3).

Compared to casein fed mice, the mRNA expression of SREBP1c was significantly decreased in whey fed mice. The mRNA expression levels of ACCα and FAS were also significantly decreased in whey protein fed mice (Figure 4).

### 3.4. Epididymal Adipose Tissue

In the epididymal adipose tissue of whey protein fed mice, the mRNA expression
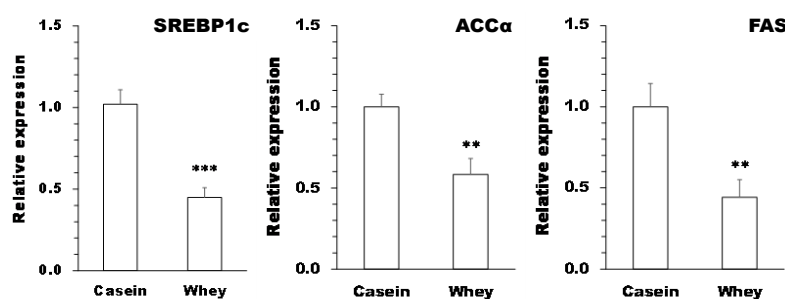
Figure 2. Effect of whey protein on mRNA levels of the enzymes related to fatty acid synthesis in the gastrocnemius muscle. The mRNA expression levels were normalized to the casein control. **: $P < 0.01$, ***: $P < 0.001$, Ɨ: $P < 0.01$.
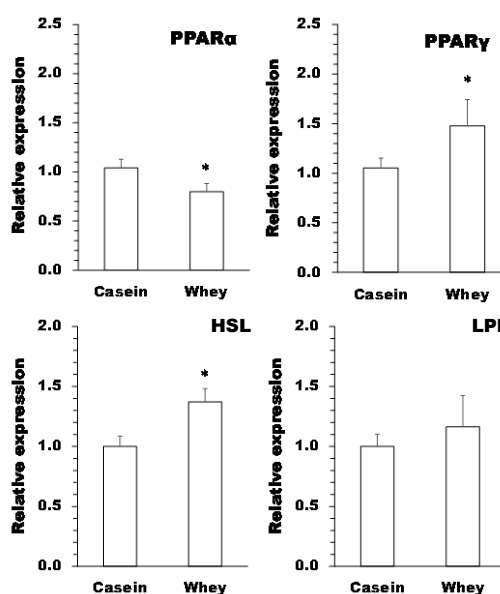
Figure 3. Effect of whey protein on mRNA levels of the enzymes related to triglyceride breakdown in the liver. The mRNA expression levels were normalized to the casein control. Ɨ: $P < 0.1$, *: $P < 0.05$.
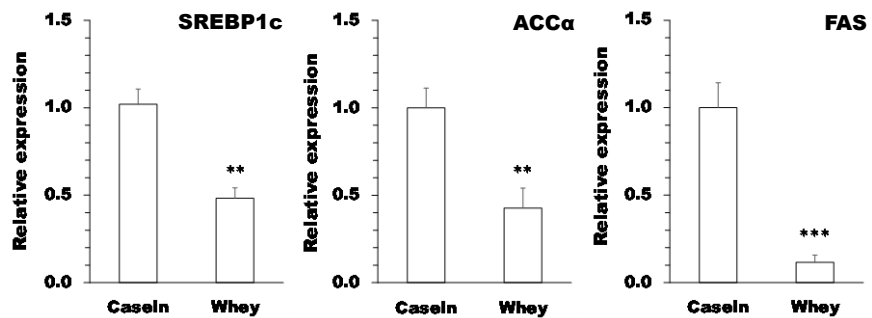
Figure 4. Effect of whey protein on mRNA levels of the enzymes related to fatty acid synthesis in the liver. The mRNA expression levels were normalized to the casein control. **: $P < 0.01$, ***: $P < 0.001$.

of PPARγ was significantly increased while that of PPARα was not changed. The mRNA expression of HSL was significantly increased by whey protein, and that of LPL was not significantly changed (Figure 5).

The mRNA expression of SREBP1c decreased. A significant decrease in the mRNA expression levels of FAS was observed, while that of ACCα was not changed (Figure 6).

4. Discussion

In this study, the gene expression patterns of the transcription factors, PPARs and SREBP1c, and their downstream enzymes related to lipid metabolism in the gastrocnemius muscle, liver and epididymal adipose tissue of mice fed whey protein, were analyzed.

PPARα is a transcription factor that regulates lipid metabolism by modulating the gene expression of enzymes related to energy production from triglyceride [16] [17] [18]. For example, PPARα upregulates the gene expression of the two major enzymes for triglyceride breakdown, HSL and LPL. In this study, it was shown that PPARα was transcriptionally upregulated by whey protein in the gastrocnemius muscle. Consequently, the gene expression of LPL was also upregulated. These results suggest that the supply of fatty acids by triglyceride breakdown is enhanced in the gastrocnemius muscle probably for energy production.

In the liver and epididymal adipose tissue, PPARγ, not PPARα, was observed to be transcriptionally upregulated, suggesting that the regulatory mechanisms for lipid metabolism might be different between the gastrocnemius muscle and the liver and adipose tissue of mice fed whey protein. In this study, it was observed that the expression levels of PPARγ and HSL in the liver were concomitantly increased. In the epididymal adipose tissue also, PPARγ was observed to be transcriptionally upregulated by whey protein together with HSL, coinciding with the report that the expression of HSL was enhanced by PPARγ in adipose tissue of rosiglitazone-treated mice [19]. The same expression pattern of PPARγ and HSL might indicate that similar, if not identical, metabolic configuration for
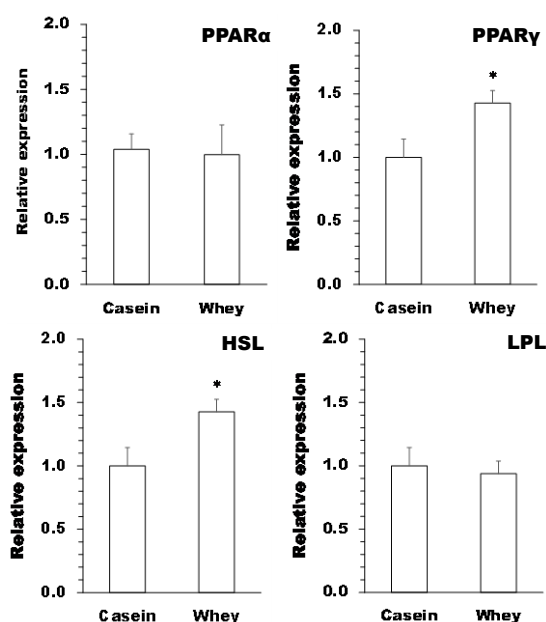
Figure 5. Effect of whey protein on mRNA levels of the enzymes related to triglyceride breakdown in the epididymal adipose tissue. The mRNA expression levels were normalized to the casein control. *: P < 0.05.
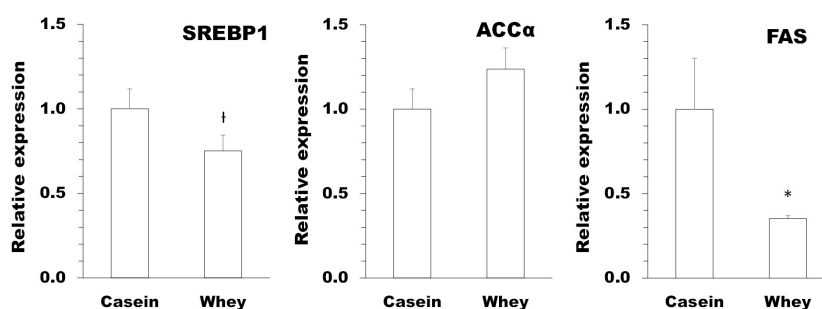
Figure 6. Effect of whey protein on mRNA levels of the enzymes related to fatty acid synthesis in the epididymal adipose tissue. The mRNA expression levels were normalized to the casein control. ǂ: P < 0.1, *: P < 0.05.

triglyceride breakdown takes place in the liver and epididymal adipose tissue of mice fed whey protein.

Major enzymes in the pathway of fatty acid synthesis are ACCα, which catalyzes the conversion of acetyl CoA to malonyl CoA, and FAS, which catalyzes the synthesis of palmitic acid from acetyl CoA and malonyl CoA. These enzymes are transcriptionally regulated by the transcriptional factor, SREBP1c [20] [21]. The results in this study indicate that the gene expression of SREBP1c was suppressed in the gastrocnemius muscle and liver by whey protein, subsequently lowering the expression levels of ACCα and FAS. In the epididymal adipose tissue, the expression of SREBP1c tended to be decreased less potently than those in the other two tissues. These results implicate that regulation modes of fatty acid synthesis vary between tissues. That is, the fatty acid synthesis in the gastrocnemius muscle and liver of mice fed whey protein might be more efficiently

suppressed, whereas the adipose tissue might be more potently directed toward fatty acid synthesis than other tissues.

The food component, resveratrol, modulates lipid metabolism by activating PPARs [22] [23] [24]. Resveratrol directly interacts with PPARs and enhance their transcriptional activity [25]. It has also been reported that soy protein decreases blood glucose and triglyceride levels via the modulation of PPARα pathways [26] and suppresses the expression of genes related to lipid synthesis by downregulating the gene expression of SREBP1c [27]. Although these food components and whey protein share similar properties with respect to modulating PPARs and SREBP1c, only whey protein shows potency to stimulate protein synthesis, differentiating it from resveratrol and soy protein.

Beneficial properties of whey protein, a protein source with balanced amino acid ratios, abilities to stimulate muscle protein synthesis and suppress inflammatory reactions, suggests its potential use in the prevention of sarcopenia or delay of its progress. The results of this study might add a new aspect to the properties of whey protein. Namely, whey protein modulates lipid metabolism directing toward enhancement of triglyceride breakdown and suppression of fatty acid synthesis.

This property might help patients with sarcopenia especially with a morbidity of obesity improve lipid metabolism by directing it toward lipid catabolism. On the other hand, this property might not disturb usage of whey protein for patients with sarcopenia without a morbidity of obesity, because it is thought that whey protein, as a dairy food component, moderately modulates lipid metabolism in these patients without adverse outcomes. All of these properties of whey protein might become an incentive to promote its therapeutic usage for sarcopenia with and without a morbidity of obesity.

5. Conclusion

The current study demonstrated that whey protein intake affected the mRNA expression of the transcription factors, PPARα, PPARγ and SREBP1c, in the gastrocnemius muscle, liver and epididymal adipose tissue of mice, concomitantly affecting the expression of their downstream enzymes, HSL, LPL, ACCα and FAS in these tissues. The expression patterns of these factors and enzymes indicated that whey protein transcriptionally modulated lipid metabolism directing it toward enhancement of triglyceride breakdown and suppression of fatty acid synthesis. Together with beneficial properties of whey protein to enhance protein synthesis and to suppress inflammation, these results implicate that whey protein may be a potent candidate for dietary therapies for sarcopenia and sarcopenia obesity.

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Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

References

[1] Volek, J.S., Volk, B.M., Gómez, A.L., Kunces, L.J., Kupchak, B.R., Freidenreich, D.J., Aristizabal, J.C., Saenz, C., Dunn-Lewis, C., Ballard, K.D., Quann, E.E., Kawiecki, D.L., Flanagan, S.D., Comstock, B.A., Fragala, M.S., Earp, J.E., Fernandez, M.L., Bruno, R.S., Ptolemy, A.S., Kellogg, M.D., Maresh, C.M. and Kraemer, W.J. (2013) Whey Protein Supplementation during Resistance Training Augments Lean Body Mass. *Journal of the American College of Nutrition*, **32**, 122-135.
https://doi.org/10.1080/07315724.2013.793580

[2] Cribb, P.J., Williams, A.D., Stathis, C.G., Carey, M.F. and Hayes, A. (2007) Effects of Whey Isolate, Creatine, and Resistance Training on Muscle Hypertrophy. *Medicine and Science in Sports and Exercise*, **39**, 298-307.
https://doi.org/10.1249/01.mss.0000247002.32589.ef

[3] Dangin, M., Guillet, C., Garcia-Rodenas, C., Gachon, P., Bouteloup-Demange, C., Reiffers-Magnani, K., Fauquant, J., Ballèvre, O. and Beaufrère, B. (2003) The Rate of Protein Digestion Affects Protein Gain Differently during Aging in Humans. *Journal of Physiology*, **549**, 635-644. https://doi.org/10.1113/jphysiol.2002.036897

[4] Bodine, S.C., Stitt, T.N., Gonzalez, M., Kline, W.O., Stover, G.L., Bauerlein, R., Zlotchenko, E., Scrimgeour, A., Lawrence, J.C. and Glass, D.J. (2001) Akt/mTOR Pathway Is a Crucial Regulator of skeletal Muscle Hypertrophy and Can Prevent Muscle Atrophy *in Vivo*. *Nature Cell Biology*, **3**, 1014-1019.
https://doi.org/10.1038/ncb1101-1014

[5] Farnfield, M.M., Carey, K.A., Gran, P., Trenerry, M.K. and Cameron-Smith, D. (2009) Whey Protein Ingestion Activates mTOR-Dependent Signaling after Resistance Exercise in Young Men: A Double-Blinded Randomized Controlled Trial. *Nutrients*, **1**, 263-275. https://doi.org/10.3390/nu1020263

[6] Baer, D.J., Stote, K.S., Paul, D.R., Harris, G.K., Rumpler, W.V. and Clevidence, B.A. (2011) Whey Protein But Not Soy Protein Supplementation Alters Body Weight and Composition in Free-Living Overweight and Obese Adults. *Journal of Nutrition*, **141**, 1489-1494. https://doi.org/10.3945/jn.111.139840

[7] Kume, H., Okazaki, K. and Sasaki, H. (2006) Hepatoprotective Effects of Whey Protein on D-Galactosamine-Induced Hepatitis and Liver Fibrosis in Rats. *Bioscience, Biotechnology, and Biochemistry*, **70**, 1281-1285.
https://doi.org/10.1271/bbb.70.1281

[8] Kume, H., Okazaki, K., Yamaji, T. and Sasaki, H. (2012) A Newly Designed Enteral Formula Containing Whey Peptides and Fermented Milk Product Protects Mice against Concanavalin A-Induced Hepatitis by Suppressing Overproduction of Inflammatory Cytokines. *Clinical Nutrition*, **31**, 283-289.
https://doi.org/10.1016/j.clnu.2011.10.012

[9] Sugawara, K., Takahashi, H., Sugawara, T., Yamada, K., Yanagida, S., Homma, M., Dairiki, K., Sasaki, H., Kawagoshi, A., Satake, M. and Shioya, T. (2012) Effect of Anti-Inflammatory Supplementation with Whey Peptide and Exercise Therapy in Patients with COPD. *Respiratory Medicine*, **106**, 1526-1534.
https://doi.org/10.1016/j.rmed.2012.07.001

[10] Kaido, T., Ogura, Y., Ogawa, K., Hata, K., Yoshizawa, A., Yagi, S. and Uemoto, S. (2012) Effects of Post-Transplant Enteral Nutrition with an Immunomodulating Diet Containing Hydrolyzed Whey Peptide after Liver Transplantation. *World Journal of Surgery*, **36**, 1666-1671. https://doi.org/10.1007/s00268-012-1529-9

[11] Dalle, S., Rossmeislova, L. and Koppo, K. (2017) The Role of Inflammation in Age-Related Sarcopenia. *Frontiers in Physiology*, **8**, 1045-1061.

https://doi.org/10.3389/fphys.2017.01045

[12] Umegaki, H. (2015) Sarcopenia and Diabetes: Hyperglycemia Is a Risk Factor for Age-Associated Muscle Mass and Functional Reduction. *Journal of Diabetes Investigation*, **6**, 623-624. https://doi.org/10.1111/jdi.12365

[13] Pani, G., Cavallucci, V. and Bartoccioni, E. (2016) Age-Related Sarcopenia: Diabetes of the Muscle? *Journal of Clinical and Molecular Endocrinology*, **1**, 29-30.

[14] Kreidieh, D., Itani, L., El Masri, D., Tannir, H., Citarella, R. and El Ghoch, M. (2018) Association between Sarcopenic Obesity, Type 2 Diabetes, and Hypertension in Overweight and Obese Treatment-Seeking Adult Women. *Journal of Cardiovascular Development and Disease*, **5**, 51-58. https://doi.org/10.3390/jcdd5040051

[15] Varma, V., Yao-Borengasser, A., Rasouli, N., Nolen, G.T., Phanavanh, B., Starks, T., Gurley, C., Simpson, P., McGehee, R.E.Jr., Kern, P.A. and Peterson, C.A. (2009) Muscle Inflammatory Response and Insulin Resistance: Synergistic Interaction between Macrophages and Fatty Acids Leads to Impaired Insulin Action. *American Journal of Physiology: Endocrinology and Metabolism*, **296**, E1300-E1310. https://doi.org/10.1152/ajpendo.90885.2008

[16] Aoyama, T., Peters, J.M., Iritani, N., Nakajima, T., Furihata, K., Hashimoto, T. and Gonzalez, F.J. (1998) Altered Constitutive Expression of Fatty Acid-Metabolizing Enzymes in Mice Lacking the Peroxisome Proliferator-Activated Receptor α (PPARα). *Journal of Biological Chemistry*, **273**, 5678-5684. https://doi.org/10.1074/jbc.273.10.5678

[17] Kersten, S., Seydoux, J., Peters, J.M., Gonzalez, F.J., Desvergne, B. and Wahli, W. (1999) Peroxisome Proliferator-Activated Receptor α Mediates the Adaptive Response to Fasting. *Journal of Clinical Investigation*, **103**, 1489-1498. https://doi.org/10.1172/JCI6223

[18] Schoonjans, K., Peinado-Onsurbe, J., Lefebvre, A.M., Heyman, R.A., Briggs, M., Deeb, S., Staels, B. and Auwerx, J. (1996) PPARα and PPARγ Activators Direct a Distinct Tissue-Specific Transcriptional Response via a PPRE in the Lipoprotein Lipase Gene. *EMBO Journal*, **15**, 5336-5348. https://doi.org/10.1002/j.1460-2075.1996.tb00918.x

[19] Shen, W.J., Yu, Z., Patel, S., Jue, D., Liu, L.F. and Kraemer, F.B. (2011) Hormone-Sensitive Lipase Modulates Adipose Metabolism through PPARγ. *Biochimica et Biophysica Acta*, **1811**, 9-16. https://doi.org/10.1016/j.bbalip.2010.10.001

[20] Osborne, T.F. (2000) Sterol Regulatory Element-Binding Proteins (SREBPs): Key Regulators of Nutritional Homeostasis and Insulin Action. *Journal of Biological Chemistry*, **275**, 32379-32382. https://doi.org/10.1074/jbc.R000017200

[21] Eberlé, D., Hegarty, B., Bossard, P., Ferré, P. and Foufelle, F. (2004) SREBP Transcription Factors: Master Regulators of Lipid Homeostasis. *Biochimie*, **86**, 839-848. https://doi.org/10.1016/j.biochi.2004.09.018

[22] Inoue, H., Jiang, X.F., Katayama, T., Osada, S., Umesono, K. and Namura, S. (2003) Brain Protection by Resveratrol and Fenofibrate against Stroke Requires Peroxisome Proliferator-Activated Receptor Alpha in Mice. *Neuroscience Letters*, **352**, 203-206. https://doi.org/10.1016/j.neulet.2003.09.001

[23] Tsukamoto, T., Nakata, R., Tamura, E., Kosuge, Y., Kariya, A., Katsukawa, M., Mishima, S., Ito, T., Iinuma, M., Akao, Y., Nozawa, Y., Arai, A., Namura, S., Inoue, H. and Vaticanol, C.A. (2010) Resveratrol Tetramer Activates PPARα and PPARβ/δ *in Vitro* and *in Vivo*. *Nutrition and Metabolism*, **7**, 46-53. https://doi.org/10.1186/1743-7075-7-46

[24] Takizawa, Y., Nakata, R., Fukuhara, K., Yamashita, H., Kubodera, H. and Inoue, H.

(2015) The 4'-Hydroxyl Group of Resveratrol Is Functionally Important for Direct Activation of PPARα. *PLoS ONE*, **10**, e0120865. https://doi.org/10.1371/journal.pone.0120865

[25] Calleri, E.., Pochetti, G., Dossou, K.S.S., Laghezza, A., Montanari, R., Capelli, D., Prada, E., Loiodice, F., Massolini, G., Bernier, M. and Moaddel, R. (2014) Resveratrol and Its Metabolites Bind to PPARs. *ChemBioChem*, **15**, 1154-1160. https://doi.org/10.1002/cbic.201300754

[26] Yamada, Y., Muraki, A., Oie, M., Kanegawa, N., Oda, A., Sawashi, Y., Kaneko, K., Yoshikawa, M., Goto, T., Takahashi, N., Kawada, T. and Ohinata, K. (2012) Soymorphin-5, a Soy-Derived μ-Opioid Peptide, Decreases Glucose and Triglyceride Levels through Activating Adiponectin and PPARα Systems in Diabetic KKAy Mice. *American Journal of Physiology-Endocrinology and Metabolism*, **302**, E433-E440. https://doi.org/10.1152/ajpendo.00161.2011

[27] Hashidume, T., Sasaki, T., Inoue, J. and Sato, R. (2011) Consumption of Soy Protein Isolate Reduces Hepatic SREBP-1c and Lipogenic Gene Expression in Wild-Type Mice, But Not in FXR-Deficient Mice. *Bioscience, Biotechnology, and Biochemistry*, **75**, 1702-1707. https://doi.org/10.1271/bbb.110224