Journal of Life Science **2009** Vol. 19. No. 4. 423~428 ©JLS / ISSN 1225-9918

AMP-activated Kinase Regulates Adipocyte Differentiation Process in 3T3-L1 Adipocytes Treated with Selenium

Song Yi Park, Jin-Taek Hwang[1], Yun-Kyoung Lee[2], Young-Min Kim and Ock Jin Park[2]*

Department of Biological Sciences, Hannam University Daedeok Valley Campus, 461-6 Jeonmin-dong, Yuseong-gu, Daejeon 305-811, Korea
[1]Korea Food Research Institute, Functional Food Reserch center, San 46-1, Baekhyon-dong, Bundang-ku, Seongnam, Gyungki-do 463-746, Korea
[2]Department of Food and Nutrition, Hannam University Daedeok Valley Campus, 461-6 Jeonmin-dong, Yuseong-gu, Daejeon 305-811, Korea

Received November 28, 2008 / Accepted April 13, 2009

Selenium was investigated using human origin preadipocytes to see whether it possesses preventive or therapeutic effects for obesity. Unveiling the potential of selenium in the reduction of adipogenesis can help predict the therapeutic capabilities of selenium in obesity. In the present study, the molecular mechanism of the inhibition of adipogenesis by selenium was explored to unravel the involvement of the AMP-activated protein kinase. There is emerging evidence that AMPK, a sensor of cellular energy status, is a possible molecular target of controlling adipocyte differentiation on the basis of discovery that AMPK is responsible for the major metabolic responses to exercise, and integration of nutritional and hormonal signals to modulate feeding behavior or energy expenditure in the hypothalamus. Treatment of selenium resulted in inhibition of the adipocyte differentiation process and induction of mature apoptosis in 3T3-L1 adipocytes. We hypothesized that selenium may exert anti-adipogenic potential though modulating AMPK. We have found that selenium significantly activated AMPK and phosphorylated its substrate acetyl-CoA carboxylase (ACC-serine^{79}) during the inhibitory process of adipocytes. Also, the inhibition process of adipocyte differentiation by selenium was comparable to either reveratrol or a synthetic AMPK activator, AICAR (5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside). To evaluate the involvement of AMPK in anti-lipogensis, we applied AICAR and Compound C, an AMPK inhibitor, to 3T3-L1-adipocytes and found that AMPK is required for the adipocyte differentiation blocking process. These results suggest that selenium has a potential to control adipogenesis and that this effect is mediated by AMPK, an essential kinase for both inhibition of adipocyte differentiation and apoptosis of mature adipocytes.

Key words : Selenium, AMP-activated protein kinase, adipocyte differentiation, 3T3-L1-preadipocytes, 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside

Introduction

Selenium, an essential trace nutrient, has a variety of functions in cells including antioxidant properties. Dietary selenium supplementation has received a great deal of attention for its possible beneficial effects [1,2]. Additionally, selenium has been shown to have anti-tumorigenic abilities, and other metabolic regulatory functions such as glucose homeostasis and lipid concentrations [3,4]. Although numerous studies on functional aspects of selenium for the prevention of degenerative diseases have been carried out, anti-adipogenic effect of selenium has not been explored. Identifying molecular basis for controlling adipocytes has an implication for therapeutic modalities of obesity development and obesity related metabolic consequences. The modulation of AMP-activated kinase (AMPK) has emerged as an important target for the prevention and treatment of atherosclerosis as well as potentially for obesity therapy [5,6]. AMPK, a sensor of cellular energy status, emerges as a possible molecular candidate of controlling adipocyte differentiation [7]. Recently, controlling obesity and diabetes through the modulation of AMPK has gained a great deal of attention, because there is a general consensus that the major metabolic responses to exercise are mediated through AMPK [8]. Furthermore, considering increased use of selenium as a dietary supplement, it needs to be clarified the possible biological function of selenium on adipocytes. The aim of the present study was to evaluate the effects of selenium on adipocyte differentiation and induction of apoptosis through the activation of AMPK signaling. Our results show that selenium activates AMPK, inhibits adipocyte differentiation comparable to resveratrol or AICAR, and induces apoptosis of adipocytes.

***Corresponding author**
Tel : +82-42-629-8793, Fax : +82-42-629-8789
E-mail : ojpark@hnu.kr

Materials and Methods

Cell culture and reagents

3T3-L1 cells (ATCC, Manassas, VA) were cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum at 37°C in a 5% CO_2 atmosphere. Specific antibodies recognizing the phosphorylated forms of AMPK Thr^{172}, ACC Ser^{79}, β-actin were purchased from Cell Signaling Technology (Danvers, MA). Insulin, 3-isobutyl-1-methylxanthine (IBMX), and dexamethasone were from Sigma-Aldrich (St. Louis, MO). Sodium selenate was also purchased from Sigma-Aldrich.

Adipocyte differentiation

3T3-L1-preadipocyte cells were grown in 12-well plates, and adipocyte differentiation was induced for two days with hormonal mixtures containing 1M dexamethasone, 5 g/ml insulin, and 0.5 mM IBMX. After two days, the medium was changed to the normal medium containing insulin (5 g/ml).

Oil Red O staining

After completing differentiation, the cells were fixed with 3.5% formaldehyde for 20 min, and then the differentiated cells were stained with Oil Red O dye (Sigma-Aldrich).

Protein extract and Western blotting

The cells were washed with phosphate-buffered saline (PBS), scraped into lysis buffer (50mM Tris–HCl [pH 7.4], 1% NP-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EDTA, 1 mM PMSF, 1 mM sodium orthovanadate, 1 mM NaF, 1 g/ml aprotinin, 1 g/ml leupeptin, and 1 g/ml pepstatin), and subjected to Western blot analysis with specific antibodies.

Chromatin staining with Hoechst 33342

Cells were stained with 10 μM Hoechst 33342 dye (Sigma-Aldrich) for 30 min, and then cells were fixed with 3.7% para-formaldehyde for 15 min. After PBS (phosphoate-buffered saline) washing, the fluorescence intensity was assessed by fluorescence microscope.

Results

Inhibition of adipocyte differentiation was observed in 3T3–L1 adipocytes treated with selenium

We evaluated the effects of selenium on the inhibition of adipocyte differentiation. Cultured 3T3-L1 adipocytes were exposed to selenate at different doses (at day 0), and cell differentiation was performed with a hormonal cocktail contained medium. At day 6, differentiations were ended and droplets of lipid were detected by oil staining. The treatment of adipocytes with selenate markedly suppressed adipocyte differentiation (Fig. 1). These results suggest that selenium might be an efficient blocker of adipocyte differentiation, and therefore, have a potential of anti-obesity agent.

Selenium also induced mature adipocyte apoptosis

The apoptotic ability of mature adipocytes were next evaluated, and 3T3-L1 adipocytes were differentiated with hormonal cocktail treatment, and mature cells were exposed to selenium. After treatment, apoptotic pattern was observed with hoechst 33342 dye, and the apoptotic bodies were found in selenium-exposed mature adipocytes (Fig. 2A).

Selenium was found to activate AMPK in the process of adipocyte differentiation blockage

We investigated the involvement of AMPKα in the process of selenium-induced inhibition of adipocyte differentiation. AMPK activation and its substrate acetyl-CoA carboxylase (ACC) phosphorylation were examined. The AMPK activation was observed directly by examining the increase of phosphorylated AMPK and indirectly by observing the phosphorylation level of acetyl-CoA carboxylase $serine^{79}$, the best-characterized phosphorylation site by the activated AMPK. The activation of AMPKα following selenium treatment (100-400 μM) was noticeable as shown in Fig. 2B.

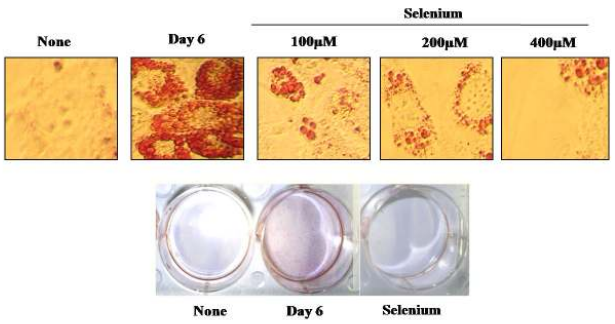

Fig. 1. The effects of selenium on 3T3-L1 adipocyte differentiation. Postconfluent 3T3-L1 cells were differentiated with hormonal mixtures in the absence or presence of selenium (100-400 μM) for 6 days. The morphological changes were photographed after Oil-Red O staining.

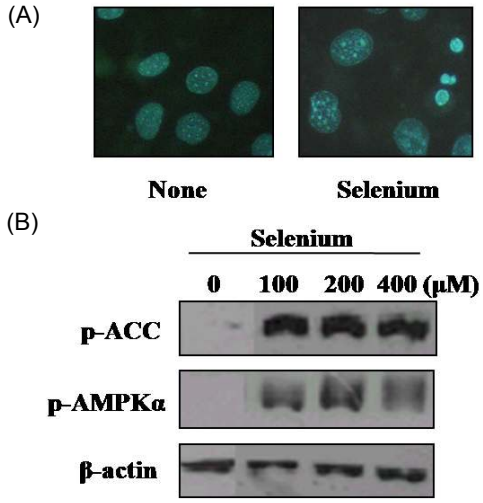

Fig. 2. Selenium activates AMPK and induces apoptosis in differentiated 3T3-L1 adipocytes. Postconfluent 3T3-L1 cells were differentiated with hormonal mixtures in the absence or presence of selenium for 6 day. Then cells were incubated with 10 μM Hoechst 33342 and fixed with 3.7% formaldehyde and fluorescence microscopic images were obtained (A). The levels of phospho-AMPKα and phospho-Acc were determined by Western blot analysis.

Blockage of adipocyte differentiation by selenium was comparable to the adipocyte differentiation inhibition by either resveratrol or AICAR

The inhibition of adipocyte differentiation of AMPK by selenium was comparable to the treatment with grape phytochemical resveratrol or AICAR, a synthetic AMPK activator in 3T3-L1 adipocytes (Fig. 3).

The involvement of AMPK activation in adipocyte differentiation process was evaluated with AICAR and Compound C

As shown in Fig. 4, the treatment of 3T3-L1 adipocytes with AICAR (1 mM and 2 mM) and Compound C, an AMPK inhibitor, revealed that AICAR activated AMPK, and the activation of AMPK by AICAR was inhibited by co-treatment with Compound C. AICAR, a synthetic form of AMPK activator, has shown to be an inhibitor of cell anabolism, especially in cancerous cells. This result indicates that AMPK is involved in the inhibition process of adipocyte differentiation.

Discussion

Correct assessment of the underlying mechanisms of anti-obesity effect of dietary agent is inevitable in considering

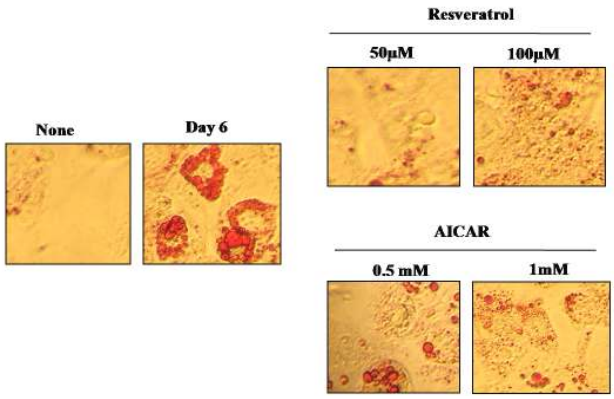

Fig. 3. The effects of resveratrol and AICAR on 3T3-L1 adipocyte differentiation. Postconfluent 3T3-L1 cells were differentiated with hormonal mixtures in the absence or presence of resveratrol (50, 100 μM) and AICAR (0.5, 1 mM) for 6 days. The morphological changes were photographed after Oil-Red O staining.

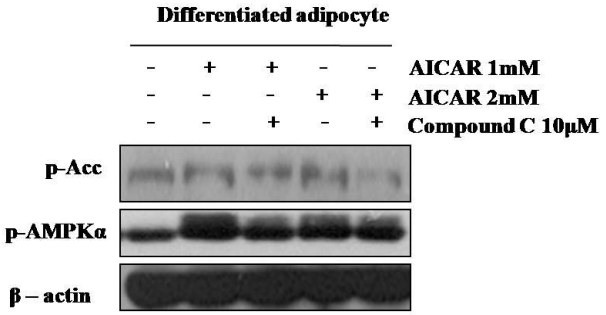

Fig. 4. The role of AMPKα in differentiated adipocytes. Postconfluent 3T3-L1 cells were differentiated with hormonal mixtures for 6day. After differentiation, cells were pretreated with Compound C (10 μM) for 30 min and treated with AICAR (1, 2 mM) for 6 hr. The levels of phospho-AMPKα and phospho-Acc were determined by Western blot analysis.

the application to human intervention trials. In the present study the molecular basis of selenium with emphasis on their ability to control intracellular signaling cascades of AMP activated kinase (AMPK) responsible for the inhibition of adipogenesis was investigated. The evolutionarily conserved serine/threonine kinase, AMPK, is known as a primary cellular homeostasis sensor and effecter modulating energy balance and a possible target molecule of anti-obesity [9]. Hypothalamic AMPK was found to integrate nutritional and hormonal signals modulating feeding behavior and energy expenditure [10]. Moreover, adipocyte-derived hormones such as leptin and adiponectin are shown to activate AMPK [11,12]. The mechanism by which affects AMPK regulation with physiological stimuli or anti-obesity agents

might present a promising target for the development of strategies for the treatment of obesity. In present study, we investigated the effects of selenium on adipocyte differentiation in relation to AMPK activation. We have observed that adipogenesis was induced during adipocyte differentiation by hormonal cocktail, and selenium significantly inhibited the process of adipocyte differentiation and led to mature adipocyte apoptosis. The previous report from our laboratory has shown the possibility of ROS as an upstream signal of AMPK in the process of adipocyte control by the naturally occurring compounds such as genistein, EGCG (epigallocatechin-3-gallate) and capsaicin [13]. From the present study it was suggested that the activation of AMPK was necessary for the inhibition of adipogenesis in 3T3-L1 cells, and AMPK is a novel and critical component of both inhibition of adipocyte differentiation and apoptosis of mature adipocyte by selenium implying AMPK as a prime target of obesity control. Obesity is considered to be resulting from the imbalance between energy intake and energy expenditure that may be linked to a pathologic overgrowth of adipose cells [22]. The mass of adipose tissue is thought to be modulated by the blocking of adipogenesis from precursors, and the control of the size of mature adipocytes. Therefore, obesity is caused by the hypertrophy of adipocytes as well as the recruitment of new adipocytes from precursor cells, and these two processes are critical for the adipocyte differentiation [22]. AMPK is known to play a major role in energy homeostasis by coordinating adaptive responses in ATP-depleting metabolic states of exercise [8]. Furthermore, the persistent activation of AMPK showed to be connected to p53-dependent cellular senescence suggesting its role as an intrinsic regulator of the cell cycle in mammalian cells [14]. Recently, AMPK cascades have emerged as novel targets for the treatment of obesity and type 2 diabetes. AMPK is known to be activated with 5-amino-imidazole-4-carboxamide riboside (AICAR), which is converted to a nucleotide that mimics the effect of AMP, and the long term treatment with AICAR prevented the development of diabetes in animal models [15]. Also, the proapoptotic potential of the activated was observed in the AMPK over-expressed conditions of various cells [16,17]. Our results show that selenium activates AMPK, blocks adipocyte differentiation comparable to resveratrol or AICAR, and induces apoptosis of adipocytes. The anti-proliferatory and lipolytic effects of selenium have been attributed to their ability to modulate various signaling pathways, specially, the control of cell proliferation and survival. However, the precise target of their anti-proliferatory effect remained unresolved. Here, we introduce AMPK as a possible main target of these compounds in their anti-obesity activity. AMPK is activated by various stimuli including exercise, heat shock and ROS [15]. The activated for blocks the anabolic pathways and promotes catabolic pathway, and thus activation of AMPK is linked to inhibition of cell proliferation and apoptosis. AMPK is known to be involved in the oxidative stress-induced cellular apoptosis through the inhibition of specific protein trans-elongation factor 2 when it is phosphorylated [18,19]. The more recent studies suggest that AMPK plays a critical role in the inhibition of cellular protein synthesis as well as stress-induced apoptosis [20]. It has been reported that AMPK activation is necessary for the therapeutic effect of metformin and troglitazone [23]. The mechanism by which affects AMPK regulation with physiological stimuli or anti-obesity agents might present a promising target for the development of strategies for the treatment of obesity. AMPK cascades have been postulated to respond to the intracellular level of AMP or AMP:ATP ratio, and to be highly sensitive to the oxidative stress [21]. The exact mechanism to stimulate preadipocyte mitosis and differentiation in vivo remains exclusive. However, it is proposed that hypertrophy of fat cells grown beyond a certain size might propagate to differentiate by sending specific signals. Adipocyte inducers stimulate preadipocytes to undergo mitotic clonal expansion before transcriptional activation of adipocyte genes before anchoring adipocyte phenotypes [24]. The results indicated that adipogenesis was induced during adipocyte differentiation by hormonal cocktail, and selenium inhibited the adipocyte differentiation of lead to apoptosis of mature adipocytes. Also we have tested whether AICAR has the similar effect on adipocyte differentiation and AMPK activation in comparision with selenium, and we have found that selenium and AICAR inhibited the differentiation and early clonal expansion of pre-adipocytes. We have also found that AMPK inhibitor Compound C could block the inhibitory effect of AMPK on adipocyte differentiation. These results strongly suggested that the activation of AMPK was necessary for the inhibition of adipogenesis in 3T3-L1 cells, and AMPK is a novel and critical component in adipogenesis, also AMPK is necessary for the inhibition of both adipocyte differentiation and apoptosis of mature adipocyte by selenium and resveratrol implying its involvement in dietary agent-inhibited adipogenesis.

Acknowledgement

This work was supported by Hannam University Fund 2009.

References

1. Bays, H. E., J. M. Gonzalez-Campoy, G. A. Bray, A. E. Kitabchi, D. A. Bergman, A. B. Schorr, H. W. Rodbard, and R. R. Henry. 2008. Pathogenic potential of adipose tissue and metabolic consequences of adipocyte hypertrophy and increased visceral adiposity. *Expert. Rev. Cardiovasc. Ther.* **6**, 343-368.
2. Block, K. I., A. C. Koch, M. N. Mead, P. K. Tothy, R. A. Newman, and C. Gyllenhaal. 2008. Impact of antioxidant supplementation on chemotherapeutic toxicity: a systematic review of the evidence from randomized controlled trials. *Int. J. Cancer.* **123**, 1227-1239.
3. Browne, G. J., S. G. Finn, and C. G. Proud. 2004. Stimulation of the AMP-activated protein kinase leads to activation of eukaryotic elongation factor 2 kinase and to its phosphorylation at a novel site, serine 398. *J. Biol. Chem.* **279**, 12220-12231.
4. Cherukuri, D. P. and M. A. Nelson. 2008. Role of reactive oxygen species (ROS) and JNKs in selenite-induced apoptosis in HepG2 cells. *Cancer. Biol. Ther.* **7**, 689-696.
5. Dagon, Y., Y. Avraham, and E. M. Berry. 2005. AMPK activation regulates apoptosis, adipogenesis, and lipolysis by eIF2alpha in adipocytes. *Biochem. Biophys. Res. Commun.* **340**, 43-47.
6. Daval, M., F. Foufelle, and P. Ferre. 2006. Functions of AMP-activated protein kinase in adipose tissue. *J. Physiol.* **574**, 55-62.
7. Erbayraktar, Z., O. Yilmaz, A. T. Artmann, R. Cehreli, and C. Coker. 2007. Effects of selenium supplementation on antioxidant defense and glucose homeostasis in experimental diabetes mellitus. *Biol. Trace. Elem. Res.* **118**, 217-226.
8. Gao, S., K. P. Kinzig, S. Aja, K. A. Scott, W. Keung, S. Kelly, K. Chohnan, S. Strynadka, W. W. Smith, K. L. Tamashiro, E. E. Ladenheim, G. V. Tu, Y. Ronnett, M. J. Birnbaum, G. D. Lopaschuk, and T. H. Moran. 2007. Leptin activates hypothalamic acetyl-CoA carboxylase to inhibit food intake. *Proc. Natl. Acad. Aci. USA* **104**, 17358-17363.
9. Gray, S. L. and A. J. Vidal-Puig 2007. Adipose tissue expandability in the maintenance of metabolic homeostasis. *Nutr. Rev.* **65**, 7-12.
10. Hardie, D. G., D. Carling, and M. Carlson 1998. The AMP-activated/SNF1 protein kinase subfamily: metabolic sensors of the eukaryotic cell? *Annu. Rev. Biochem.* **67**, 821-855.
11. Horman, S., G. Browne, U. Krause, J. Patel, D. Vertommen, L. Bertrand, A. Lavoinne, L. Hue, C. Proud, and M. Rider. 2002. Activation of AMP-activated protein kinase leads to the phosphorylation of elongation factor 2 and an inhibition of protein synthesis. *Curr. Biol.* **12**, 1419-1423.
12. Huypens, P., E. Quartier, D. Pipeleers, and M. Van de Casteele. 2005. Metformin reduces adiponectin protein expression and release in 3T3-L1 adipocytes involving activation of AMP activated protein kinase. *Eur. J. Pharmacol.* **518**, 90-95.
13. Hwang, J. T., I. J. Park, J. I. Shin, Y. K. Lee, S. K. Lee, H. W. Baik, J. Ha, and O. J. Park. 2005. Genistein, EGCG, and capsaicin inhibit adipocyte differentiation process via activating AMP-activated protein kinase. *Biochem. Biophys. Res. Commun.* **338**, 694-696.
14. Joens, R. G., D. R. Plas, S. Kubek, M. Buzzai, J. Mu, Y. Xu, M. J. Birnbaum, and C. B. Thompson. 2005. AMP-activated protein kinase induces a p-53 dependent metabolic checkpoint. *Mol. Cell* **18**, 283-293.
15. Kemp, B. E., D. Stapleton, D. J. Campbell, Z. P. Chen, S. Murthy, M. Walter, A. Gupta, J. J. Adams, F. Katsis, B. van Denderen, I. G. Jennings, T. Iseli, B. J. Michell, and L. A. Witters. 2003. AMP-activated protein kinase, super metabolic regulator. *Biochem. Soc. Trans.* **31**, 162-168.
16. Kola, B. 2008. Role of AMP-activated protein kinase in the control of appetite. *J. Neuroendocrinol.* **20**, 942-951.
17. Kola, B., A. B. Grossman, and M. Korbonits. 2008. The role of AMP-activated protein kinase in obesity. *Front. Horm. Res.* **36**, 198-211.
18. Kubota, N., W. Yano, T. Kubota, T. Yamauchi, S. Itoh, H. Kumagai, H. Kozono, I. Takamoto, S. Okamoto, T. Shiuchi, R. Suzuki, H. Satoh, A. Tsuchida, M. Moroi, K. Sugi, T. Noda, H. Ebinuma, Y. Ueta, T. Kondo, E. Araki, O. Ezaki, R. Nagai, K. Tobe, Y. Terauchi, K. Ueki, Y. Minokoshi, and T. Kadowaki. 2007. Adiponectin stimulates AMP-activated protein kinase in the hypothalamus and increases food intake. *Cell Metab.* **6**, 55-68.
19. MacDougald, O. A. and S. Mandrup. 2002. Adipogenesis: forces that tip the scales. *Trends. Endocrinol. Metab.* **13**, 5-11.
20. Meisse, D., M. Van de Casteele, C. Beauloye, I. Hainault, B. A. Kefas, M. H. Rider, F. Foufelle, and L. Hue. 2002. Sustained activation of AMP-activated protein kinase induces c-Jun N-terminal kinase activation and apoptosis in liver cells. *FEBS. Lett.* **526**, 38-42.
21. Mukherjee, P., T. J. Mulrooney, J. Marsh, D. Blair, T. C. Chiles, and T. N. Seyfried. 2008. Differential effects of energy stress on AMPK phosphorylation and apoptosis in experimental brain tumor and normal brain. *Mol. Cancer* **7**, 37-51.
22. Shimomura, I., R. E. Hammer, J. A. Richardson, S. Ikemoto, Y. Bashmakor, J. L. Goldstein, and M. S. Brown. 1998. Insulin resistance and diabetes mellitus in transgenic mice expressing nuclear SREBP-1c in adipose tissue: model for congenital generalized lipodystrophy. *Genes Dev.* **12**, 3182-3194.
23. Song, X. M., M. Fiedler, D. Galuska, J. W. Ryder, M. Fernstrom, A. V. Chibalin, H. Wallberg-Henriksson, and J. R. Zierath. 2002. 5-Amino-imidazole-4-carboxamide ribonucleoside treatment improves glucose homeostasis in insulin-resistant diabetic (ob/ob) mice. *Diabetologia.* **45**, 56-65.
24. Sorisky, A. 1999. From pre-adipocyte to adipocyte: differentiation-directed signals of insulin from the cell surface to the nucleus. *Crit. Rev. Clin. Lab. Sci.* **36**, 1-34.

초록 : AMP-activated protein kinase가 셀레늄으로 처리된 3T3-L1 지방세포의 분화과정 억제에 관한 연구

박송이·황진택[1]·이윤경[2]·김영민·박옥진[2]*

(한남대학교 생명나노과학대학 생명과학과, [1]한국식품연구원, [2]한남대학교 생명나노과학대학 식품영양학과)

셀레늄은 필수미네랄로 항산화작용 등의 체내 주요 작용을 수행한다고 알려져 있다. 만성질환 등의 예방과 치료 등의 바람직한 효능을 기대하여 섭취가 늘고 있어서 주목을 받고 있다. 셀레늄은 암의 억제나 혈중포도당조절 및 지방억제 등의 목적으로도 이용되고 있다. 이러한 다양한 연구에도 불구하고 지방세포를 이용한 지방세포분화나 지방세포사멸 촉진에 미치는 영향은 아직 연구되어 있지 않다. 셀레늄의 지방세포조절을 통한 분자기전에 대한 연구는 늘어가고 있는 비만이나 비만관련대사질환의 조절에 학문적 근거를 제공할 수 있다. 본 연구에서는 셀레늄이 AMP-activated protein kinase (AMPK)를 중심으로 지방 미분화세포인 3T3-L1 지방세포의 분화억제 및 분화된 세포의 사멸작용의 기전을 밝히고자 하였다. AMPK는 세포 내 에너지상태의 센서로 작용하는데 실제적으로 운동의 지방대사 촉진기능이 이 단백질에 기인한다는 사실이 밝혀지면서 각광을 받고 있다. 또한 시상하부에서 AMPK는 호르몬이나 영양소 등의 생리적인 인자들을 통합하고 식욕이나 신체에너지 대사를 총괄하는 물질로 대두되고 있다. 셀레늄을 지방세포의 분화과정에 처리하였을 때에 현저한 지방세포로의 분화가 억제되었고 이미 다 분화된 성숙된 지방세포에 처리하였을 때는 세포사멸(apoptosis)을 유도하는 것을 발견하였으며 이와 동시에 AMPK의 활성화 촉진 및 AMPK의 지질인 acetyl-CoA carboxylase의 serine^{79}를 인산화하는 것으로 나타났다. 또한 이러한 셀레늄의 지방세포분화 억제 능력은 AMPK의 활성화 합성물질인 AICAR나 포도의 폴리페놀인 레스베라트롤의 능력과 같은 정도의 억제능을 보여주었다. 또한 AMPK의 항 지방축적작용에서의 필수성을 알아보기 위하여 지방세포에 AMPK 활성화 물질인 AICAR와 억제물질인 Compound C를 사용하여 AMPK활성화를 측정하여 본 결과 지방세포분화와 지방축적의 억제과정에 있어서 AMPK가 필수적임을 밝혀내었다.