

Chicken recombinant adiponectin enhances fatty acid metabolism in oleic acid- and palmitic acid-treated LMH cells

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ABSTRACT - The objective of this study was to investigate the effects of recombinant adiponectin on chicken liver cells. The full-length chicken adiponectin gene was amplified by PCR and cloned into the vector pET-32a, followed by the transformation of the vector into *Escherichia coli* BL21. SDS-PAGE was used to detect and analyze the purity of the expressed recombinant protein. Induction was performed with 1 mM IPTG at 30 °C for 3 h, and the recombinant thioredoxin-adiponectin fusion protein was purified using Ni-NTA affinity chromatography. Chicken adiponectin was successfully expressed and purified in a bacterial system. In addition, the chicken recombinant adiponectin demonstrated that it ameliorates palmitic acid- and oleic acid-induced adipogenesis, in which an increase in β -oxidation and a decrease in lipogenesis-related genes may be involved. In summary, chicken recombinant adiponectin enhances fatty acid metabolism in LMH cells.

Keywords: chicken, fatty acid metabolism, recombinant protein

1. Introduction

Adiponectin (ApN) and its receptors exist in birds, but their composition and function may differ from those of mammals. An investigation into the function of chicken ApN could be helpful for a study on the mechanism of physiological or nutritional regulation in chickens. In mammals, ApN attenuates the metabolic dysregulation of fatty acid oxidation and glucose utilization and ameliorates insulin resistance; these functions are evident, not only in obese or in insulin-resistant animals, but also in healthy individuals (Fruebis et al., 2001; Yamauchi et al., 2002). However, we have limited information on whether ApN exerts a similar physiological function in poultry.

ApN is a cytokine-type molecule that requires binding to specific receptors to express its function. ApN receptor 1 and ApN receptor 2 have been cloned from several species, including mouse, pig, and chicken (Yamauchi et al., 2003; Ding et al., 2004; Yuan et al., 2006; Ramachandran et al., 2007). Additionally, its receptors are ubiquitously expressed in various tissues. In mice, the most abundant expression of AdipoR1 is in muscle tissue, with a relative high expression of AdipoR2 in liver tissue (Yamauchi et al., 2003). Different forms of ApN have various affinities with their receptors, and they can exert different biological functions in peripheral tissues (Yamauchi et al., 2002). For chickens, the gene encoding ApN consists of 735 nucleotides and has more than 65% homology compared with mammals (Maddineni et al., 2005; Yuan et al., 2006). The cDNA of the receptors AdipoR1 and AdipoR2 also have close to 80% homology with those of mammals (Ramachandran et al., 2007).

ApN in chickens is predominantly present in the form of macromolecules or high molecular weight (HMW) adiponectin, with a molecular weight that is greater than 669 kDa (Hendricks et al., 2009).

In addition, our previous research also confirmed that HMW ApN was highly correlated with age in a positive fashion in both sexes (Chen et al., 2021). Although we know some characteristics of chicken ApN, current research does not have an in-depth understanding of its physiological functions. Therefore, the production of recombinant proteins for the investigation of the features of chicken ApN would be an appropriate direction for providing new insights into the adipokine modulation of physiological function in avian species. Studies on the effect of fatty acids-induced hepatocyte steatosis have demonstrated that palmitic acid (PA) and oleic acid (OA) mixtures treatment induced steatosis is associated with apoptosis in hepatocytes cultures (Feldstein et al., 2003). The OA or PA have also been reported to have many health disadvantages and lipotoxicity (Chou et al., 2014; Geethangili et al., 2021). To elucidate the chicken adiponectin signaling on hepatocyte, we investigated the effects of fatty acid metabolism in PA- and OA-treated cells.

2. Material and Methods

2.1. Construction of plasmids

The reference sequence of the chicken ApN gene was obtained from GenBank, under the reference number NM_206991.1. The full-length sequence of the chicken ApN gene is shown in Figure 1. The coding region of the ApN gene for its insertion into the vector pET32a was amplified by PCR using the oligonucleotides (forward: 5'-GACGACGACGACAAGGCCATGAG GGGCTCAGTAGGCTTC-3'; reverse: 5'-GCTCGAGTGCGGCCGCAA GCTTGACGGTCATCTGTGTCTGGGTA-3'), which introduced a *Hind*III (Cat. #1060A, Takara Bio, Shiga, Japan) site at the 3' end. The amplified ApN gene fragment was inserted into the vector pET32a using the In-Fusion HD enzyme (Cat. #638909, Takara Bio, Shiga, Japan), resulting in the plasmid named pET32a-adiponectin. All of the constructed plasmids were transformed into *E. coli* DH5 α and confirmed by PCR and DNA sequencing.

2.2. Bacterial transformation

Competent cells were removed from -80 °C and thawed on ice, then 5 μ L of the plasmid DNA and 50 μ L of the competent cells were mixed gently by pipetting up and down. The mixture was placed on ice for 30 min and then transferred to a 37 °C water bath for 45 s, then put back on ice for 2 min. Twenty microliters of SOC medium were added to the bacteria and incubated in a shaking incubator at 37 °C for approximately 60 min. The cells were spread onto LB agar plates with a selection antibiotic by using a sterile spreader, and then incubated at 37 °C for 6 h.

2.3. Plasmid DNA purification

Plasmid DNA purification with the TOOLS Plasmid Mini kit (Cat. #TT-A03-3, Biotools, New Taipei City, Taiwan) is based on the alkaline lysis method, followed by the adsorption of DNA onto a silica membrane. The bacterial cells were harvested in a microcentrifuge tube by centrifugation at 12,000 rpm (~13,400 $\times g$) for 1 min at room temperature, and then all traces of supernatant were removed. Pelleted bacterial cells were resuspended in 200 μ L of buffer P1. Two hundred microliters of buffer P2 were added and mixed thoroughly by inverting the tube gently 10 times, then 300 μ L of buffer P3 were added and mixed thoroughly by inverting the tube 10 times. The solution, which became cloudy, was centrifuged at 12,000 rpm (~13,400 $\times g$) for 10 min, and a compact white pellet formed. The supernatants were applied to Spin Column CP3 by decanting or pipetting, and then centrifuged at 12,000 rpm (~13,400 $\times g$) for 1 min, then the flow-through was discarded. Spin Column CP3 was washed by adding 400 μ L of Buffer PD and centrifuging at 12,000 rpm (~13,400 $\times g$) for 1 min, and the flow-through was discarded. Spin Column CP3 was washed by adding 700 μ L of Buffer PW and centrifuging at 12,000 rpm (~13,400 $\times g$) for 1 min, and the flow-through was discarded. To remove the residual wash buffer PW, Spin Column CP3 was centrifuged at 12,000 rpm (~13,400 $\times g$) for an additional 3 min. Spin Column CP3 was placed into a clean 1.5 mL

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atgaggggctcagtaggcttctcctttgctcactgctgctggccctaagtggcacagag
M R G S V G F L L C S L L L A L S G T E 20
atggctgaccaggctgaccagtccgaccccaaaatgtcgtgtgccaaactggatgggagga
M A D Q A D Q S D P K M S C A N W M G G 40
gcacccggacaccctggccacaatgggctgcctggaagggacggcaaggatggaaaagat
A P G H P G H N G L P G R D G K D G K D 60
ggacaaaagggagacaaaggagagccaggtctacaaggtgtcaaaggggacacgggtgaa
G Q K G D K G E P G L Q G V K G D T G E 80
aaggggtgccacaggtgcagaaggaccgagaggatttccaggacacatggggatgaagggg
K G A T G A E G P R G F P G H M G M K G 100
cagaaggggtgaaagctcctacgtgtaccgctccgccttcagcgtggggctgacagagcga
Q K G E S S Y V Y R S A F S V G L T E R 120
gccccccacccaacgtccccatccgcttcaccaagatcttctacaacgagcagaaccac
A P H P N V P I R F T K I F Y N E Q N H 140
tacgacagcagcaccggcaagtctctcgcagcatccccggcacgtacttctttgcttac
Y D S S T G K F L C S I P G T Y F F A Y 160
cacctgacggctctacatgacggacgtcaaggtcagcctctacaagaaggacaaggcagtg
H L T V Y M T D V K V S L Y K K D K A V 180
atcttcacctatgaccagttccaggagaacaacgttgaccaagcgagcggctctgtcttg
I F T Y D Q F Q E N N V D Q A S G S V L 200
ctgcacctcagcttgggggacgaggtctggctccaggtgtacggggagggaacaacaac
L H L S L G D E V W L Q V Y G E G N N N 220
ggggctctatgctgacaacatcaatgattccacttccatgggcttctcctgtaccagac
G V Y A D N I N D S T F M G F L L Y P D 240
acagatgaccgttaa
T D D R - 244

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The chicken adiponectin cDNA contains 735 bp, which translates into a protein that is 244 amino acids in length.

Figure 1 - Full-length sequence of chicken adiponectin gene.

microcentrifuge tube. To elute the DNA, 50–100 μ L of Buffer EB or water (pH 7.0–8.5) were added to the center of each spin column (CP3), stood for 2–5 min, and centrifuged at 12,000 rpm (\sim 13,400 \times g) for 2 min. The collected DNA was stored at 4 or -20 $^{\circ}$ C.

2.4. Protein expression and purification

The presence of the gene insertion was verified using DNA sequencing, and then the plasmid was transformed into *E. coli* BL21 (Cat. #69450-3CN, Novagen, Madison, USA). A single colony was selected for the inoculation of 200 mL of LB medium, which contained 100 μ g/mL ampicillin. For induction, isopropyl- β -D-thiogalactoside (IPTG, Cat. #N714-10ML, Amresco LLC, Solon, USA) was added to the culture to a final concentration of 1 mM, and simultaneously supplemented with 100 μ g/mL ampicillin (Cat. #A9518, Sigma, St. Louis, USA). The culture was further incubated at 30 $^{\circ}$ C with shaking for an additional 3 h. The culture medium was centrifuged at 5000 rpm for 20 min, and the induced bacterial pellet was harvested and stored at -20 $^{\circ}$ C. For recombinant protein purification, the bacterial pellet was suspended in 20 mL crude extract buffer (50 mM Tris-HCl,

100 mM NaCl, 10 mM imidazole, and 1 μ M phenylmethanesulfonyl fluoride, pH 7.5), and then broken up using JY88-IIN sonication (Scientz, Ningbo, China). The supernatant fraction with soluble protein from the lysate was collected via centrifugation at 10,000 rpm for 20 min. The supernatant was loaded onto 0.5 mL Ni-NTA His-Tag Purification Resin (Cyrusbioscience, New Taipei City, Taiwan) that had been pre-equilibrated with lysis buffer (50 mM Tris-HCl and 100 mM NaCl, pH 7.5) containing 10 mM imidazole, and incubated on ice at 300 Mot 1/min for 1 h to enable optimal binding between the fusion protein and the resin. The mix of supernatant and resin was poured into Poly-Prep[®] prepacked columns (Cat. #731-1550, Bio-Rad, Hercules, USA). After washing the column with 1 mL lysis buffer containing 10 mM imidazole to remove the unbound protein, the bounded protein was eluted with increasing concentrations of imidazole (50, 125, 250, and 500 mM) in 1.5 mL of lysis buffer, and the fractions were collected and kept at 4 °C.

2.5. SDS-PAGE

Eight microliters of the 4 \times reducing sample buffer (0.25 M Tris-HCl, 8% SDS, 30% glycerol, 0.02% Bromophenol Blue, and 0.3 M DTT, pH 6.8) was added to 24 μ L of protein samples (a total volume of 32 μ L), then boiled under reducing conditions in the 1.5-mm-thick gel, which was cast with a 12.5% separating and a 4% stacking gel mixture, and loaded with 2 μ L Precision Plus Protein[™] Dual Color Standards (Cat. #161-0374, Bio-Rad, Hercules, USA) and 30 μ L aliquots of each sample. The electrophoresis was performed in a Mini-PROTEAN Tetra Cell (Cat. #1658006FC, Bio-Rad, Hercules, USA) filled with appropriately 1 \times running buffer at 100 V for 30 min, and then at 180 V for approximately 1 h, until the blue line almost reached the bottom of the gel. To visualize the protein bands and determine the purity and molecular weight of the protein, the gels were stained with Coomassie Brilliant Blue R-250 (Cat. #CO-3360, W. S. Simpson, Hertfordshire, England) and shaken at room temperature for 30–60 min until the bands were visible. The staining solution was then decanted at room temperature on a shaker until the desired background was achieved.

2.6. Cell culture and treatment

The chicken hepatocellular carcinoma cell line, LMH (CRL-2117, ATCC) was cultured in Waymouth's MB 752/1 medium (Biological Industries, Cromwell, CT, USA) with 10% fetal bovine serum (Thermo Fisher Scientific, Waltham, MA, USA) and 1% 100 units/mL penicillin, 100 mg/mL streptomycin, and 0.025 mg/mL amphotericin B (Biological Industries, Kibbutz Beit Haemek, Israel). LMH cells were incubated in 6-well plates at an initial density of 5×10^5 /well, in a 5% CO₂ humidified atmosphere incubator at 37 °C. When the cells reached 95% confluence, they were starved with serum-free Waymouth's MB 752/1 medium for 8 h. For the fatty acid treatment, PA (Cayman Chemical Company, Ann Arbor, MI, USA) and OA (Cayman Chemical Company, Ann Arbor, MI, USA) were both dissolved in ethanol (Sigma-Aldrich Corporation, St. Louis, MO, USA), and the dissolved PA or OA were aliquoted and stored at -20 °C. The 200 μ M PA and 200 μ M OA were conjugated with 1% BSA with low fatty acid, low endotoxin, and low IgG (US Biological Life Sciences, Swampscott, MA, USA) in complete Waymouth's MB 752/1 medium, with sonication for at least 30 min prior to the treatment of the cells. The BSA-conjugated OA and PA were added to the wells for 48 h, with or without recombinant ApN.

2.7. RNA extraction and gene expression analyses

Total RNA from the LMH cells was extracted using GENEzol[™] Reagent (Geneaid Biotech, Ltd., New Taipei City, Taiwan). The RNA concentration was measured at 260/280 nm using a NanoDrop[™] One (Thermo Fisher Scientific, Waltham, MA, USA). For each reverse-transcribed sample, 7 μ g RNA were treated with TURBO[™] DNase (Thermo Fisher Scientific, Waltham, MA, USA) to remove the genomic DNA. The DNase-treated total RNA was used to generate cDNA using a High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, Waltham, MA, USA). Quantitative real-time PCR reactions

were performed via a StepOnePlus™ Real-Time PCR System (Applied Biosystems, Foster City, CA, USA), with a DyNAmo Flash SYBR Green High-ROX Detection Kit (Finnzymes, Espoo, Finland). The conditions of the program were as follows: 2 min at 95 °C for polymerase activation, 40 cycles at 95 °C for 5 s for denaturation, and 60 °C for 30 s for annealing/extension. The specific primer sequences are listed in Table 1.

Table 1 - Primer sequences used for real-time PCR analysis

Gene	Forward primer	Reverse primer
β-actin	5'-gtgatggactctggtgatgg-3'	5'-ccatgtgtcctggaatcct-3'
SREBP1c	5'-gccctctgtgaccttcttctc-3'	5'-actcagccatgatgcttcttc-3'
FAS	5'-aagcaattcgtcacggacag-3'	5'-ggcaccatcaggactaagca-3'
ACC	5'-agtctgattgagcatggca-3'	5'-ctccagatggcgtagattc-3'
CPT1α	5'-ctgggtattgccacgaagc-3'	5'-gccatggctaaggttttcgt-3'
PPARα	5'-acggagtccaatcgc-3'	5'-aaccttacaaccttcacaa-3'

2.8. Statistical analysis

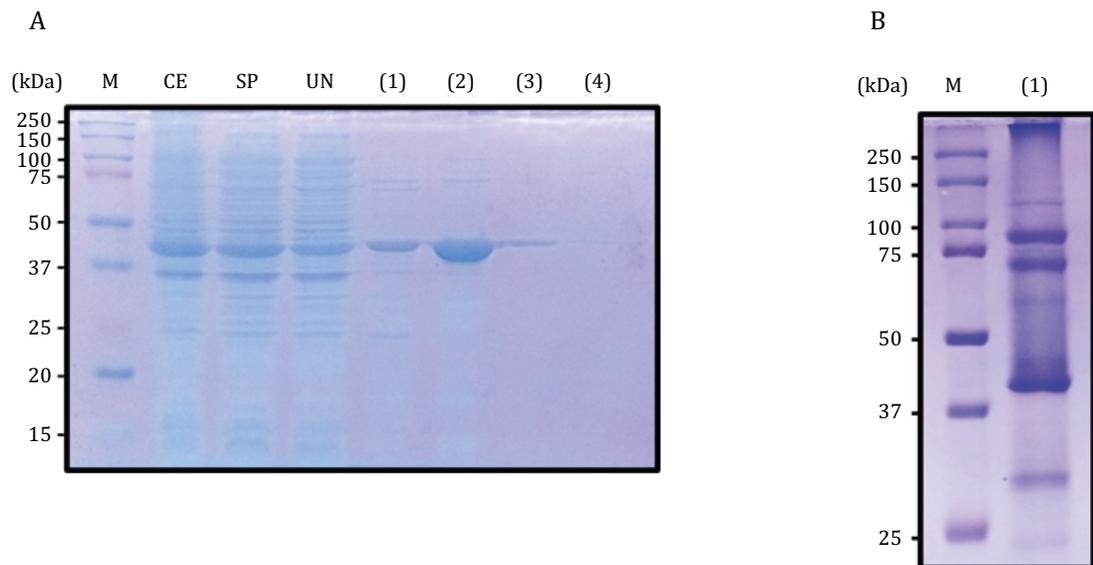
Statistical analyses were performed using GraphPad software (version 5 for Windows). The collected data were tested via one-way ANOVA, and the mean differences were compared using Tukey's multiple comparison test. Significance was declared at $P \leq 0.05$.

3. Results

3.1. Expression and purification of chicken ApN in *E. coli*

To construct the combination of an optimized expression vector with bacterial expression systems that were adapted for high-yield recombinant protein expression, the ApN coding sequence was cloned into the pET32a vector using the *NcoI* and *HindIII* restriction sites. The antibiotic resistance gene-selected vectors were transformed into competent DH5α cells for storage and scale-up applications. Colony PCR reactions were performed to screen the clones, and the PCR products with the correct product sizes were sequenced for verification. All of the sequence alignments of the cloned ApN fusions displayed 100% similarity with the sequence of chicken ApN published in GenBank.

The *E. coli* host strain BL21, transformed with the expression vector pET32a-adiponectin, produced a recombinant fusion protein of approximately 45 kDa after IPTG induction. Trx-adiponectin containing a (His)₆-tag between the fusion partners Trx-tag and ApN was purified using Ni-NTA His-Tag Purification Resin. The recombinant protein was eluted with four lots of 1.5 mL volumes of buffer containing 50, 125, 250, and 500 mM imidazole. The purified recombinant protein was collected, and its purity was assessed using SDS-PAGE. Most of the recombinant protein was detected at the 125 mM imidazole buffer-eluted fraction (Figure 2A), and approximately 95% of the protein consisted of the soluble fusion protein Trx-adiponectin, with a molecular mass of approximately 45 kDa, which was consistent with the size of the predicted Trx-adiponectin fusion protein. SDS-PAGE under non-reducing conditions was performed for the detection of the oligomerization process in ApN. The majority of the Trx-adiponectin fusion protein migrated as monomers at approximately 45 kDa, and as dimers of approximately 90 kDa, with a few trimers being present at approximately 135 kDa (Figure 2B). Approximately 1.5 mg of pure Trx-adiponectin was obtained from bacterial cultures, with a 1.24 g pellet, and the yield accounted for 5% of the total bacterial protein.



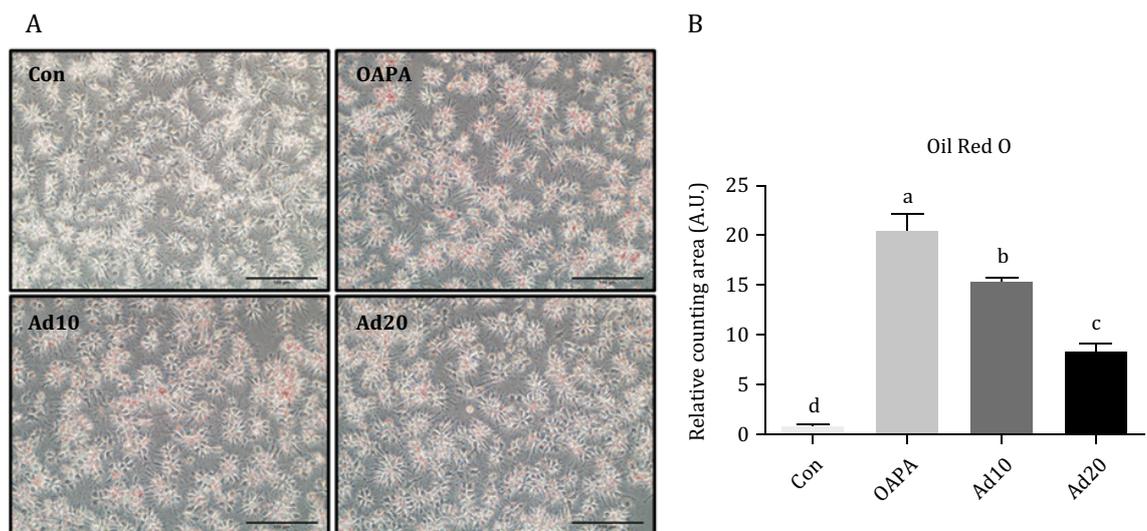
M - protein markers; CE - crude extract cell lysate; SP - soluble protein; UN - unbound protein.

A) Expression of chicken recombinant adiponectin from pET32a-adiponectin in *E. coli*. Samples were eluted using buffers containing 50 mM imidazole (lane 1), 125 mM imidazole (lane 2), 250 mM imidazole (lane 3), and 500 mM imidazole (lane 4). B) The expression profile of recombinant adiponectin via non-reducing SDS-PAGE. Samples were eluted by buffers containing 125 mM imidazole (lane 1).

Figure 2 - Expression and purification of recombinant adiponectin pET32a-adiponectin vector.

3.2. Chicken recombinant ApN retards lipid accumulation in LMH cells

Compared with the untreated cells, OA and PA treatment increased lipid accumulation in the LMH cells. Additionally, the treatment of chicken recombinant ApN (10 $\mu\text{g}/\text{mL}$ and 20 $\mu\text{g}/\text{mL}$) could ameliorate lipid accumulation in the LMH cells (Figure 3A). The quantification data from Oil Red O staining showed the same pattern as the representative figure (Figure 3B).



A) Representative images of Oil Red O staining; B) Quantification of Oil Red O staining.

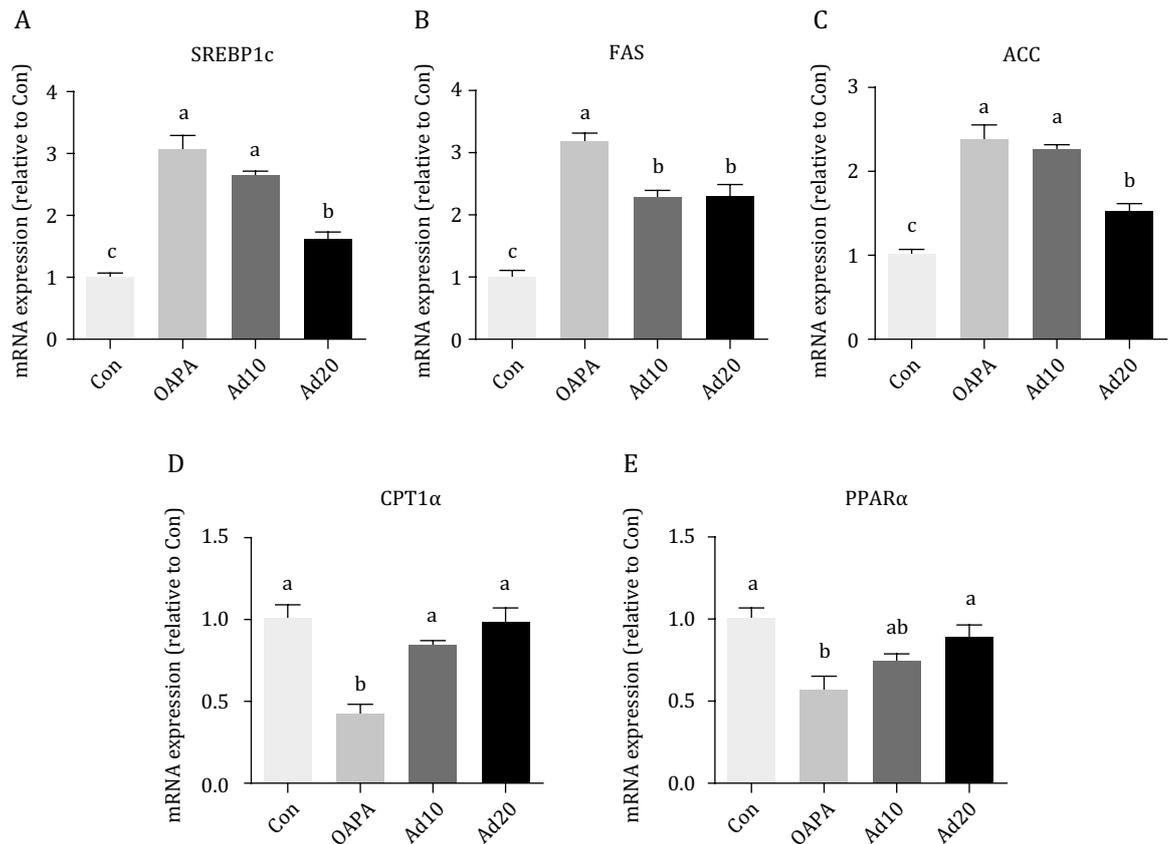
Con - control; OAPA - oleic acid and palmitic acid; Ad10 - chicken recombinant adiponectin, 10 $\mu\text{g}/\text{mL}$; Ad20 - chicken recombinant adiponectin, 20 $\mu\text{g}/\text{mL}$.

Data were expressed as relative mean \pm SEM (n = 5 in each group). Different letters indicate statistical differences.

Figure 3 - Effects of chicken recombinant adiponectin on palmitic acid and oleic acid-induced adipogenesis in LMH cells.

3.3. Effects of chicken recombinant ApN on lipid metabolism-related genes

The OA and PA challenges enhanced lipogenic genes such as SREBP1c, FAS, and ACC in LMH cells (Figure 4A, 4B, and 4C). Additionally, CPT1- α and PPAR α , which represented lipolysis-related genes, decreased with OA and PA treatment (Figure 4D and 4E). However, chicken recombinant ApN can retard the lipid accumulation caused by OA and PA treatment, through an increase in β -oxidation, and a decrease in adipogenesis.



A) SREBP1c; B) FAS; C) ACC; D) CPT1 α ; E) PPAR α .

Con - control; OAPA - oleic acid and palmitic acid; Ad10 - chicken recombinant adiponectin, 10 μ g/mL; Ad20 - chicken recombinant adiponectin, 20 μ g/mL.

SREBP1c - sterol regulatory element-binding transcription factor 1c; FAS - fatty acid synthase; ACC - acetyl-CoA carboxylase; CPT1 α - carnitine palmitoyltransferase 1 α ; PPAR α - peroxisome proliferator-activated receptor α .

The mRNA were determined using real-time PCR and normalized to β -actin.

Data were expressed as the relative mean \pm SEM (n = 5 in each group).

Different letters indicate statistical differences.

Figure 4 - Quantification of gene expression in chicken recombinant adiponectin-treated LMH cells.

4. Discussion

The production of recombinant ApN for the investigation of the role of chicken ApN in physiological function was one of the goals of the current study. In addition, the use of nutrients to regulate the function of ApN or ApN receptors to further improve the growth performance of chickens and the metabolic efficiency is the expected scope of application in the future.

ApN and its receptors have been well-characterized in mammalian species; non-mammalian ApN has not been specifically addressed in previous research. In general, decreased circulating ApN

concentrations are associated with insulin resistance, obesity, and type 2 diabetes (Spranger et al., 2003; Yamauchi et al., 2001). The administration of ApN to mice decreases the plasma levels of glucose, free fatty acids, and triglycerides; it increases muscle fatty acid oxidation and induces weight loss (Kadowaki and Yamauchi, 2005). Moreover, ApN-deficient mice feature insulin resistance, dyslipidemia, and hypertension (Kubota et al., 2002). Impairments in ApN multimerization lead to defects in ApN function and secretion, and are associated with obesity (Liu et al., 2008). DsbA-L is highly expressed in adipose tissue and acts as a critical factor for ApN multimerization. Its expression level is negatively correlated with obesity in mice and humans. DsbA-L activity is stimulated by the insulin sensitizer rosiglitazone in 3T3-L1 cells (Liu et al., 2008). The overexpression of DsbA-L promoted ApN multimerization while downregulating DsbA-L expression markedly, and it selectively reduced ApN secretion from 3T3-L1 cells (Liu et al., 2008). AdipoR1 and AdipoR2 serve as the major AdipoRs *in vivo*, in which AdipoR1 can activate the AMP kinase (AMPK) pathway and AdipoR2 acts on the activation of the peroxisome proliferator-activated receptor alpha (PPAR α) pathway, to enhance insulin sensitivity and to decrease inflammation (Yamauchi and Kadowaki, 2008). The disruption of both AdipoR1 and R2 exterminates ApN binding and its downstream actions, including the abolishment of ApN-induced AMPK activation, and the decreased activity of the PPAR- α signaling pathway, thereby increasing tissue triglyceride content, inflammation, oxidative stress, insulin resistance, and glucose intolerance (Yamauchi et al., 2007).

Most of the effects of ApN in mammalian liver are beneficial for enhancing energy utilization, immunomodulation, and liver injury (Lin et al., 2014; Dong et al., 2015; Wang et al., 2016; Ishtiaq et al., 2019; Ryu et al., 2021). In addition, the alleviated function of liver tissue can be achieved via PPAR signaling (Ishtiaq et al., 2019). The results shown here indicated that chicken recombinant ApN exerts functions that are similar to those in mammals, which increase fatty acid metabolism in LMH cells under OA and PA treatments. A recent study indicates that HMW ApN has a predominant role in metabolic tissues (Pajvani et al., 2004). In our previous study, chicken circulating HMW ApN and fat disulfide-bond A oxidoreductase-like protein, a regulator involved in ApN secretion, were elevated upon high-fat diet feeding. Additionally, the mRNA expression of ApN and ApN receptors, as well as additional adipose-related genes such as FAS, adipose triglyceride lipase, lipoprotein lipase, and peroxisome proliferator-activated receptor γ , also increased in chicken fat tissue under a high-fat diet challenge (Chen et al., 2018). The aforementioned reference implies that HMW ApN may also have particular functions in chickens. However, its detailed mechanisms need to be investigated in the future.

5. Conclusions

The current study demonstrated that chicken recombinant adiponectin ameliorates palmitic acid- and oleic acid-induced adipogenesis, in which an increase in β -oxidation and a decrease in lipogenesis-related genes may be involved. Therefore, chicken recombinant adiponectin enhances fatty acid metabolism in LMH cells.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: Y.-Y. Lin. Data curation: Y.-R. Zhuang and Y.-Y. Lin. Formal analysis: Y.-R. Zhuang and Y.-Y. Lin. Funding acquisition: Y.-Y. Lin. Investigation: Y.-Y. Lin. Methodology: Y.-R. Zhuang and Y.-Y. Lin. Project administration: Y.-Y. Lin. Supervision: Y.-Y. Lin. Validation: Y.-Y. Lin. Writing – original draft: Y.-R. Zhuang and Y.-Y. Lin. Writing – review & editing: Y.-Y. Lin.

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References

- Chen, C. Y.; Chen, Y. J.; Ding, S. T. and Lin, Y. Y. 2018. Expression profile of adiponectin and adiponectin receptors in high-fat diet feeding chickens. *Journal of Animal Physiology and Animal Nutrition* 102:1585-1592. <https://doi.org/10.1111/jpn.12979>
- Chen, Y. C.; Huang, T. C. and Lin, Y. Y. 2021. Expression profile of circulatory adiponectin and plasma variables in broilers. *Brazilian Journal of Poultry Science* 23:eRBCA-2020-1302. <https://doi.org/10.1590/1806-9061-2020-1302>
- Chou, I. P.; Lin, Y. Y.; Ding, S. T. and Chen, C. Y. 2014. Adiponectin receptor 1 enhances fatty acid metabolism and cell survival in palmitate-treated HepG2 cells through the PI₃ K/AKT pathway. *European Journal of Nutrition* 53:907-917. <https://doi.org/10.1007/s00394-013-0594-7>
- Ding, S. T.; Liu, B. H. and Ko, Y. H. 2004. Cloning and expression of porcine adiponectin and adiponectin receptor 1 and 2 genes in pigs. *Journal of Animal Science* 82:3162-3174. <https://doi.org/10.2527/2004.82113162x>
- Dong, Z.; Su, L.; Esmaili, S.; Iseli, T. J.; Ramezani-Moghadam, M.; Hu, L.; Xu, A.; George, J. and Wang, J. 2015. Adiponectin attenuates liver fibrosis by inducing nitric oxide production of hepatic stellate cells. *Journal of Molecular Medicine* 93:1327-1339. <https://doi.org/10.1007/s00109-015-1313-z>
- Feldstein, A. E.; Canbay, A.; Guicciardi, M. E.; Higuchi, H.; Bronk, S. F. and Gores, G. J. 2003. Diet associated hepatic steatosis sensitizes to Fas mediated liver injury in mice. *Journal of Hepatology* 39:978-983. [https://doi.org/10.1016/s0168-8278\(03\)00460-4](https://doi.org/10.1016/s0168-8278(03)00460-4)
- Fruebis, J.; Tsao, T. S.; Javorschi, S.; Ebbets-Reed, D.; Erickson, M. R. S.; Yen, F. T.; Bihain, B. E. and Lodish, H. F. 2001. Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. *Proceedings of the National Academy of Sciences* 98:2005-2010. <https://doi.org/10.1073/pnas.98.4.2005>
- Geethangili, M.; Lin, C. W.; Mersmann, H. J. and Ding, S. T. 2021. Methyl brevifolinicarbonylate attenuates free fatty acid-induced lipid metabolism and inflammation in hepatocytes through AMPK/NF- κ B signaling pathway. *International Journal of Molecular Sciences* 22:10062. <https://doi.org/10.3390/ijms221810062>
- Hendricks III, G. L.; Hadley, J. A.; Krzysik-Walker, S. M.; Prabhu, K. S.; Vasilatos-Younken, R. and Ramachandran, R. 2009. Unique profile of chicken adiponectin, a predominantly heavy molecular weight multimer, and relationship to visceral adiposity. *Endocrinology* 150:3092-3100. <https://doi.org/10.1210/en.2008-1558>
- Ishtiaq, S. M.; Rashid, H.; Hussian, Z.; Arshad, M. I. and Khan, J. A. 2019. Adiponectin and PPAR: a setup for intricate crosstalk between obesity and non-alcoholic fatty liver disease. *Reviews in Endocrine and Metabolic Disorders* 20:253-261. <https://doi.org/10.1007/s11154-019-09510-2>
- Kadowaki, T. and Yamauchi, T. 2005. Adiponectin and adiponectin receptors. *Endocrine Reviews* 26:439-451. <https://doi.org/10.1210/er.2005-0005>
- Kubota, N.; Terauchi, Y.; Yamauchi, T.; Kubota, T.; Moroi, M.; Matsui, J.; Eto, K.; Yamashita, T.; Kamon, J.; Satoh, H.; Yano, W.; Froguel, P.; Nagai, R.; Kimura, S.; Kadowaki, T. and Noda, T. 2002. Disruption of adiponectin causes insulin resistance and neointimal formation. *Journal of Biological Chemistry* 277:25863-25866. <https://doi.org/10.1074/jbc.C200251200>
- Lin, Z.; Wu, F.; Lin, S.; Pan, X.; Jin, L.; Lu, T.; Shi, L.; Wang, Y.; Xu, A. and Li, X. 2014. Adiponectin protects against acetaminophen-induced mitochondrial dysfunction and acute liver injury by promoting autophagy in mice. *Journal of Hepatology* 61:825-831. <https://doi.org/10.1016/j.jhep.2014.05.033>
- Liu, M.; Zhou, L.; Xu, A.; Lam, K. S. L.; Wetzell, M. D.; Xiang, R.; Zhang, J.; Xin, X.; Dong, L. Q. and Liu, F. 2008. A disulfide-bond A oxidoreductase-like protein (DsbA-L) regulates adiponectin multimerization. *Proceedings of the National Academy of Sciences* 105:18302-18307. <https://doi.org/10.1073/pnas.0806341105>
- Maddineni, S.; Metzger, S.; Ocón, O.; Hendricks III, G. and Ramachandran, R. 2005. Adiponectin gene is expressed in multiple tissues in the chicken: food deprivation influences adiponectin messenger ribonucleic acid expression. *Endocrinology* 146:4250-4256. <https://doi.org/10.1210/en.2005-0254>
- Pajvani, U. B.; Hawkins, M.; Combs, T. P.; Rajala, M. W.; Doebber, T.; Berger, J. P.; Wagner, J. A.; Wu, M.; Knopps, A.; Xiang, A. H.; Utzschneider, K. M.; Kahn, S. E.; Olefsky, J. M.; Buchanan, T. A. and Scherer, P. E. 2004. Complex distribution, not absolute amount of adiponectin, correlates with thiazolidinedione-mediated improvement in insulin sensitivity. *Journal of Biological Chemistry* 279:12152-12162. <https://doi.org/10.1074/jbc.M311113200>

- Ramachandran, R.; Ocón-Grove, O. M. and Metzger, S. L. 2007. Molecular cloning and tissue expression of chicken AdipoR1 and AdipoR2 complementary deoxyribonucleic acids. *Domestic Animal Endocrinology* 33:19-31. <https://doi.org/10.1016/j.domaniend.2006.04.004>
- Ryu, J.; Hadley, J. T.; Li, Z.; Dong, F.; Xu, H.; Xin, X.; Zhang, Y.; Chen, C.; Li, S.; Guo, X.; Zhao, J. L.; Leach, R. J.; Abdul-Ghani, M. A.; DeFronzo, R. A.; Kamat, A.; Liu, F. and Dong, L. Q. 2021. Adiponectin alleviates diet-induced inflammation in the liver by suppressing MCP-1 expression and macrophage infiltration. *Diabetes* 70:1303-1316. <https://doi.org/10.2337/db20-1073>
- Spranger, J.; Kroke, A.; Mohlig, M.; Bergmann, M. M.; Ristow, M.; Boeing, H. and Pfeiffer, A. F. 2003. Adiponectin and protection against type 2 diabetes mellitus. *The Lancet* 361:226-228. [https://doi.org/10.1016/S0140-6736\(03\)12255-6](https://doi.org/10.1016/S0140-6736(03)12255-6)
- Wang, H.; Zhang, H.; Zhang, Z.; Huang, B.; Cheng, X.; Wang, D.; la Gahu, Z.; Xue, Z.; Da, Y.; Li, D.; Yao, Z.; Gao, F.; Xu, A. and Zhang, R. 2016. Adiponectin-derived active peptide ADP335 exerts anti-inflammatory and anti-fibrotic activities in thioacetamide-induced liver injury. *Scientific Reports* 6:19445. <https://doi.org/10.1038/srep19445>
- Yamauchi, T.; Kamon, J.; Waki, H.; Terauchi, Y.; Kubota, N.; Hara, K.; Mori, Y.; Ide, T.; Murakami, K.; Tsuboyama-Kasaoka, N.; Ezaki, O.; Akanuma, Y.; Gavrilova, O.; Vinson, C.; Reitman, M. L.; Kagechika, H.; Shudo, K.; Yoda, M.; Nakano, Y.; Tobe, K.; Nagai, R.; Kimura, S.; Tomita, M.; Froguel, P. and Kadowaki, T. 2001. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nature Medicine* 7:941-946. <https://doi.org/10.1038/90984>
- Yamauchi, T.; Kamon, J.; Minokoshi, Y.; Ito, Y.; Waki, H.; Uchida, S.; Yamashita, S.; Noda, M.; Kita, S.; Ueki, K.; Eto, K.; Akanuma, Y.; Froguel, P.; Foufelle, F.; Ferre, P.; Carling, D.; Kimura, D.; Nagai, R.; Kahn, B. B. and Kadowaki, T. 2002. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nature Medicine* 8:1288-1295. <https://doi.org/10.1038/nm788>
- Yamauchi, T.; Kamon, J.; Ito, Y.; Tsuchida, A.; Yokomizo, T.; Kita, S.; Sugiyama, T.; Miyagishi, M.; Hara, K.; Tsunoda, M.; Murakami, K.; Ohteki, T.; Uchida, S.; Takekawa, S.; Waki, H.; Tsuno, N. H.; Shibata, Y.; Terauchi, Y.; Froguel, P.; Tobe, K.; Koyasu, S.; Taira, K.; Kitamura, T.; Shimizu, T.; Nagai, R. and Kadowaki, T. 2003. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature* 423:762-769. <https://doi.org/10.1038/nature01705>
- Yamauchi, T. and Kadowaki, T. 2008. Physiological and pathophysiological roles of adiponectin and adiponectin receptors in the integrated regulation of metabolic and cardiovascular diseases. *International Journal of Obesity* 32(Suppl 7):S13-S18. <https://doi.org/10.1038/ijo.2008.233>
- Yamauchi, T.; Nio, Y.; Maki, T.; Kobayashi, M.; Takazawa, T.; Iwabuchi, M.; Okada-Iwabuchi, M.; Kawamoto, S.; Kubota, N.; Kubota, T.; Ito, Y.; Kamon, J.; Tsuchida, A.; Kumagai, K.; Kozono, H.; Hada, Y.; Ogata, H.; Tokuyama, K.; Tsunoda, M.; Ide, T.; Murakami, K.; Awazawa, M.; Takamoto, I.; Froguel, P.; Hara, K.; Tobe, K.; Nagai, R.; Ueki, K. and Kadowaki, T. 2007. Targeted disruption of AdipoR1 and AdipoR2 causes abrogation of adiponectin binding and metabolic actions. *Nature Medicine* 13:332-339. <https://doi.org/10.1038/nm1557>
- Yuan, J.; Liu, W.; Liu, Z. L. and Li, N. 2006. cDNA cloning, genomic structure, chromosomal mapping and expression analysis of ADIPOQ (adiponectin) in chicken. *Cytogenetic and Genome Research* 112:148-151. <https://doi.org/10.1159/000087527>