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Dehydroepiandrosterone reduces accumulation of lipid droplets in primary chicken hepatocytes by biotransformation mediated *via* the cAMP/PKA-ERK1/2 signaling pathway

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ABSTRACT

Dehydroepiandrosterone (DHEA) is commonly used as a nutritional supplement to control fat deposition, but the mechanism of this action is poorly understood. In this study, we demonstrated that DHEA increased phosphorylation of AMP-activated protein kinase (p-AMPK). Elevated p-AMPK levels resulted in reduced expression of sterol regulatory element binding protein-1c, acetyl CoA carboxylase, fatty acid synthase and enhanced expression of peroxisome proliferators-activated receptor α and carnitine palmitoyl transferase-I, ultimately leading to the reduction of lipid droplet accumulation in primary chicken hepatocytes. We found that DHEA activates the cyclic adenosine 3′, 5′-monophosphate/protein kinase A - extracellular signal-regulated kinase 1/2 (cAMP/PKA-ERK1/2) signaling pathway, which regulates the conversion of DHEA into testosterone and estradiol by increasing the 17β-hydroxysteroid dehydrogenase and aromatase protein expression. Importantly, the fat-reducing effects of DHEA are more closely associated with the conversion of DHEA into estradiol than with the action of DHEA itself as an active biomolecule, or to its alternative metabolite, testosterone. Taken together, our results indicate that DHEA is converted into active hormones through activation of the cAMP/PKA-ERK1/2 signaling pathway; the fat-reducing effects of DHEA are achieved through its conversion into estradiol, not testosterone, and not through direct action of DHEA itself, which led to the activation of the p-AMPK in primary chicken hepatocytes. These data provide novel insight into the mechanisms underlying the action of DHEA in preventing fat deposition, and suggest potential applications for DHEA treatment to control fat deposition or as an agent to treat disorders related to lipid metabolism in animals and humans.

1. Introduction

Dehydroepiandrosterone (DHEA) is a naturally occurring adrenal steroid hormone that exerts preventative effects against obesity, diabetes, and atherosclerosis in humans and animals [1,2]. The beneficial effects of DHEA include reduced accumulation of fat, lower serum triacylglycerol, and regulation of lipogenesis [3–5], while the underlying mechanisms by which DHEA exerts these positive actions are poorly understood. It has been suggested that DHEA attenuates atherosclerosis *via* conversion to estrogen [6]. Pradhan et al. reported that aromatase-induced conversion of DHEA to sex steroids, such as estrogen, mediates metabolism in songbird brain tissue [7]; it is likely that some positive effects of DHEA can be attributed to the conversion of DHEA to biologically active steroids.

DHEA can be converted into active estrogens or androgens in peripheral tissues [8]; this transformation is dependent upon the activity of steroidogenic enzymes in target tissues [9]. We previously demonstrated that DHEA can be converted into testosterone and estradiol in rats [10,11]. As a steroid hormone precursor, DHEA is rapidly converted into androstenedione by 3β-hydroxysteroid dehydrogenase (3β-HSD), and is subsequently converted to testosterone by 17β-hydroxysteroid dehydrogenase (17β-HSD) or to estradiol by aromatase [12]. The conversion of DHEA occurs in a wide range of tissues including kidney, brain, gonads and liver [13]. Although DHEA conversion depends on the expression level of steroidogenic enzymes in target tissues, the mechanisms regulating the expression of these enzymes are not well known.

Extracellular signal-regulated kinases (ERKs) are members of the mitogen-activated protein kinase (MAPK) family, and are implicated in regulating cellular proliferation and differentiation [14]. It has been suggested that the ERK signaling pathway plays a critical role in steroidogenesis [15,16]. We have previously reported that activation of the

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ERK1/2 signal pathway results in the conversion of DHEA into active steroids in primary Leydig cells [17]; it is not known if DHEA conversion in other tissues is associated with activation of the ERK1/2 signaling pathway.

Although it is well-established that DHEA reduces fat deposition in mammals [18], the molecular mechanisms are not well understood, and little is known regarding the effects of DHEA in broiler chickens. We have previously reported that DHEA reduced fat deposition in broiler chickens and during embryonic development [19,20]; whether DHEA exerts its effects indirectly after conversion into active steroid hormones in peripheral tissues or directly as a neurosteroid remains to be elucidated. Estradiol improves lipid metabolism and prevents obesity in postmenopausal women [21], and hormone replacement therapy with estradiol regulates lipid metabolism and prevents obesity, metabolic syndrome, and diabetes [22,23]. Therefore, we hypothesized that DHEA exerts its fat-reducing effects through biotransformation to active steroid hormones in broiler chicken.

In poultry, the liver is the most important metabolic organ [24]. In hepatocytes, AMP-activated protein kinase (AMPK) acts as both a "sensor" and a "regulator" of energy [25]. AMPK can be stimulated by metabolic signals, hormones, and cytokines to regulate lipid metabolism in hepatocytes [26]. AMPK modulates lipid metabolism by regulating transcription factors related to lipid metabolism, including peroxisome proliferator activated receptor-α (PPARα) and sterol regulatory element binding protein-1c (SREBP-1c), which in turn regulate the expression of lipid metabolism enzymes [27]. DHEA has been shown to stimulate the AMPK signaling pathway in animal studies [18]. Additionally, treatment of rat cardiac myocytes with DHEA decreased intracellular energy by activating AMPK α2, and DHEA treatment increased AMPK α Thr^{172} the phosphorylation levels in mouse ovarian tissue [28]. Cyclic adenosine 3′, 5′-monophosphate (cAMP) is an important component of the kinase cascade, and connects many extracellular signals to a host of cellular pathways. The cAMP and MAPK signaling pathways are highly conserved, tightly regulated, and act through a variety of downstream mediators to regulate physiological functions in cells [29]. We have previously reported that DHEA regulates hepatic gene expression related to lipid metabolism by activating the cAMP/PKA signaling pathway in primary chicken hepatocytes [30]. We therefore hypothesized that DHEA reduces fat accumulation in broiler chicken by activating the AMPK signaling pathway.

In this study, we examined whether DHEA protects against fat deposition in primary chicken hepatocytes by conversion into active steroid hormones and whether the fat-reducing effects are associated with activation of AMPK. We also evaluated if DHEA biotransformation is associated with modulation of the cAMP/PKA-ERK1/2 signaling pathways. Our results elucidate the biochemical mechanism underlying the regulation of fat deposition by DHEA, and support its use as a nutritional supplement to control obesity-related diseases.

2. Materials and methods

2.1. Reagents

DHEA, estradiol, testosterone, dimethyl sulfoxide (DMSO), penicillin-streptomycin, transferrin, and trypsin were purchased from Sigma (St Louis, MO, USA). L-glutamine and HEPES were obtained from Amresco (Solon, OH, USA). Medium 199 and trypsin were purchased from Gibco (Grand Island, NY, USA). TRIZOL Reagent Kits were purchased from Invitrogen (Carlsbad, CA, USA). M-MLV reverse transcriptase, RNase inhibitor, and dNTP mixture were obtained from Promega (Madison, WI, USA). Ex Taq DNA polymerase was purchased from TaKaRa Bio Inc. (Shiga, Japan). SYBR Green PCR Master Mix was purchased from Roche (Basel, Switzerland).

Rabbit anti-17β-HSD and anti-aromatase antibodies were purchased from Abcam (Abcam, USA). Rabbit anti-AMPKα, phospho-AMPKα, ACCα, phospho-ACCα, ERK1/2, phospho-ERK1/2 and β-actin were purchased from Cell Signaling Technology (Boston, MA, USA). Goat anti-rabbit IgG horseradish peroxidase conjugate was purchased from Boster (Wuhan, China). U0126-EtOH (phospho-ERK1/2 inhibitor), Forskolin (PKA activator), H89 (PKA inhibitor), Trilostane (3β-HSD inhibitor), Flutamide (androgen receptor [AR] antagonist), and Fulvestrant (estrogen receptor [ER] antagonist) were purchased from Selleck Chemicals (Houston, TX, USA). 12-O-tetradecanoyl phorbol-13-acetate (TPA, an ERK activator) was purchased from ApexBio (Houston, USA). Protein assay kits were obtained from the Beyotime Biotechnology Institute (Shanghai, China). DHEA Enzyme-linked immunosorbent assay (ELISA) kits were purchased from Hengyuan Biological Technology Co. (Shanghai, China). Testosterone and estradiol radioimmunoassay (RIA) kits were purchased from the Beijing Beifang Biotechnology Institution (Beijing, China). DHEA, estradiol, or testosterone were dissolved in DMSO and stored at −20 °C. The working solutions of DHEA, estradiol, or testosterone containing of DMSO were diluted in M199 medium, and the DMSO content in all working solutions did not exceed 0.1% by volume.

2.2. Isolation of hepatocytes

Fertilized chicken eggs purchased from a commercial hatching factory and incubated at 37 °C with a relative humidity of 60%. Culture of primary embryonic hepatocytes was conducted according to the method established by Kennedy et al. [31]. Embryos were sacrificed at day 9; liver tissue was harvested under sterile conditions and washed with cold phosphate-buffered saline (PBS). Liver tissue was washed in M199 medium and then was aseptically minced into small fragments (~1 mm^3) and suspended in fresh medium for 1–2 min; the resulting supernatant was aspirated. Liver tissue was placed in a solution of PBS, lacking Ca^{2+} and Mg^{2+}, and containing 0.25 mg/mL trypsin and incubated in a vibrating water-bath (90 cycles/min at 37 °C) for 10–15 min. At the same time, the suspension was regularly pipetted to facilitate tissue dissociation, until the aggregates within the suspension consisted of 3–5 cells each. Hepatocytes were collected by centrifugation (1000 rpm for 5 min) and were filtrated through a 150-μm mesh. Hepatocytes were washed three times with fresh M199 medium, and the cell number was determined using a hemacytometer. Cell viability was determined by Trypan blue exclusion assay; hepatocytes collected in this manner regularly displayed viability exceeding 90% [32].

2.3. Primary culture of chicken hepatocytes

Primary chicken hepatocytes were seeded in monolayers on 6-well or 96-well plastic culture plates (Corning, USA) at a density of 2×10^6 cells per well in 2 mL (6-well plate) or 1×10^5 cells per well in 100 μL (96-well plate) serum-free M199 medium with 5 mg/mL transferrin, 2 mM glutamine and 1.75 mM HEPES. The culture medium also contained 100 IU/mL penicillin and 100 μg/mL streptomycin. Hepatocytes were cultured at 37 °C in an atmosphere of 95% air and 5% CO_2.

2.4. Cell viability assay

Cell viability was measured using 3-(4,5-dimethylthiozol-2-yl)-2,5 diphenyl tetrazolium bromide (MTT) reagent (Sigma; St Louis, MO, USA) as previously described by Ding et al. [33]. Primary chicken hepatocytes were cultured in 96-well plates and treated with 0, 0.1, 1, 10, 50 or 100 μM DHEA in dimethyl sulfoxide (DMSO) for 24 h prior to assessment of cell viability by the MTT assay. The absorbance of the formazan produced during the MTT assay was measured at 490 nm using a 550 Microplate Reader instrument (Bio-Rad, California, USA).

2.5. Measurement of triglyceride content

Primary chicken hepatocytes were cultured in 6-well plates (2×10^6 cells per well) and treated with 0, 0.1, 1, 10, 50 or 100 μM

DHEA in DMSO for 24 h. Hepatocytes were harvested and triglyceride (TG) content was analyzed using commercial assay kits from the Jiancheng Biotechnology Institution (Nanjing, China). Results were normalized to the protein content of each sample, as determined using a BCA assay kit (Beyotime Institute of Biotechnology, China).

2.6. Oil Red O staining

Primary chicken hepatocytes were cultured in 6-well plates (2×10^6 cells per well) and treated with 0, 0.1, 1, 10, 50 or 100 μM DHEA in DMSO for 24 h. Hepatocytes were fixed with 10% buffered formalin for at least 30 min, were incubated with 60% isopropanol for 15 min at room temperature, and were stained with Oil Red O solution for an additional 15 min. Cells were then washed four times with deionized water and were allowed to air dry. Hepatocytes were stained with hematoxylin for 5 min following Oil Red O staining in order normalize for cell number. Slides were photographed with an optical microscope (Olympus BX53; Tokyo, Japan). Twenty representative photographs were randomly selected from each group and ten independent pictures of each photo were analyzed to determine the counts and area of the lipid droplets, using Image-pro Plus 6.0 software (Media Cybernetics, Silver Spring, MD, USA).

2.7. Detection of testosterone and estradiol content by radioimmunoassay (RIA)

Primary chicken hepatocytes were cultured in 6-well plates (2×10^6 cells per well) and treated with 0, 0.1, 1, 10, 50 or 100 μM DHEA in DMSO for 24 h. Following incubation, cells were harvested, ultrasonically disrupted on ice, and centrifuged at 2500 ×*g* for 10 min at 4 °C. The supernatants were collected, and concentrations of testosterone and estradiol were determined using RIA kits, according to the manufacturer's instructions.

2.8. Detection of steroid hormone receptor content by ELISA

Primary chicken hepatocytes were cultured in 6-well plates (2×10^6 cells per well) and treated with 0, 0.1, 1, 10, 50 or 100 μM DHEA in DMSO for 20 min, or treated with 100 μM DHEA for varying lengths of time. After incubation, culture supernatant was quickly removed, and the steroid hormone receptor content was determined using an ELISA kit, according to the manufacturer's instructions (Jiancheng Biotechnology Institution, China). Results were normalized to the protein content of each sample, as determined using a BCA assay kit (Beyotime Institute of Biotechnology, China).

2.9. Real-time quantitative PCR (qPCR)

Extraction of total RNA and cDNA reverse transcription were performed according to previously described methods [34]. Briefly, total RNA was extracted from primary chicken hepatocytes using TRIZOL reagent (Invitrogen, USA), according to the manufacturer's instructions, and 2 μg total RNA from each sample was reverse transcribed into cDNA using a Superscript II kit (Promega, USA), according to the manufacturer's instructions. Aliquots of each cDNA sample were mixed with 20 μL SYBR Green PCR Master Mix (Roche, Switzerland) in the presence of 10 pmol each of forward and reverse primers for *β-actin* (used as an internal control), acetyl CoA carboxylase (*ACC*), fatty acid synthase (*FAS*), sterol regulatory element binding protein-1c (*SREBP-1c*), peroxisome proliferators-activated receptor α (*PPARα*), carnitine palmitoyl transferase-I (*CPT-I*), *AMPKα1*, *AMPKα2*, *AMPKβ1*, *AMPKβ2*, *AMPKγ1*, *AMPKγ2*, *AMPKγ3*, estrogen receptor (*ER*), and androgen receptor (*AR*) (Table 1). All samples were analyzed in duplicate using the IQ5 Sequence Detection System (Bio-Rad, California, USA). The PCR program was as follows: a hold at 95 °C for 3 min followed by 40 cycles of 95 °C for 20 s, 60 °C for 30 s, and 72 °C for 30 s. Fold changes were calculated using the $2^{-\Delta\Delta CT}$ method; the relative amount of mRNA for each target gene was determined by calculating the ratio between expression of the target mRNA and expression of *β-actin* mRNA. The primers used were designed by Primer Premier 5 (Premier Biosoft International, Palo Alto, CA, USA), and were synthesized by Invitrogen Biological Company (Shanghai, China).

2.10. Western blot analysis

Primary chicken hepatocytes were cultured in 6-well plates (2×10^6 cells per well) and treated with 0, 0.1, 1, 10, 50 or 100 μM DHEA in DMSO for 24 h. Cells were harvested by scraping and protein concentration was determined using BCA assay kits (Beyotime Institute of Biotechnology, China). The extracted proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on a 10% gel, were transferred onto PVDF membranes (Millipore, Bedford, MA, USA). Membranes were blocked for 3 h with 5% BSA in TBST and were incubated overnight with rabbit polyclonal antibodies against ERK1/2 (1:1000 dilution), p-ERK1/2 (1:1000 dilution), AMPKα (1:1000 dilution), p-AMPKα (1:1000 dilution), ACCα (1:1000 dilution), p-ACCα (1:1000 dilution), 17β-HSD (1:300 dilution), and aromatase (1:100 dilution). After washing with TBST, membranes were incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG (1:5000 dilution) in washing solution for 2 h at room temperature. Protein bands were visualized using ECL SuperSignal TM West Pico substrate (Pierce, Rock-ford, IL, USA). A monoclonal antibody against β-actin (dilution 1:1000) was used as a loading control, and all protein expression was normalized to β-actin.

2.11. Evaluation of the cAMP-PKA-ERK signaling analysis

Intracellular cyclic adenosine 3′, 5′-monophosphate assay. The accumulation of cAMP in primary chicken hepatocytes under basal or stimulated conditions was determined using an ELISA kit. Cells were cultured in 6-well plates (2×10^6 cells per well) and treated with 0, 0.1, 1, 10, 50 or 100 μM DHEA for 20 min, or were treated with 100 μM DHEA for varying lengths of time. After incubation, culture supernatant was quickly removed, and the content of cAMP was determined using an ELISA kit according to the manufacturer's instructions (Jiancheng Biotechnology Institution, China). Results were normalized to the protein content of each sample, as determined using a BCA assay kit (Beyotime Institute of Biotechnology, China).

DHEA, testosterone and estradiol content. Primary chicken hepatocytes were cultured in 6-well plates (2×10^6 cells per well). Hepatocytes were cultured for 24 h in M199 media at 37 °C, and were then pre-incubated with vehicle, 10 μM U0126, 10 μM H89, or 20 μM Forskolin for 1 h, followed by the addition of 100 μM DHEA for 1, 3, 6, 12, 24, and 48 h. The contents of testosterone and estradiol were determined using RIA kits, and the DHEA content was determined using an ELISA kit, according to the manufacturer's instructions.

ERK1/2, p-ERK1/2, 17β-HSD, and aromatase protein expression. Primary chicken hepatocytes were cultured in 6-well plates (2×10^6 cells per well). Hepatocytes were cultured for 24 h in M199 media at 37 °C, and were then pre-incubated with vehicle, 10 μM U0126, 10 μM H89, or 20 μM Forskolin for 1 h, followed by the addition of 0 μM or 100 μM DHEA for 24 h. After treatment, the protein expression of total ERK1/2, p-ERK1/2, 17β-HSD, and aromatase was determined by western blot. In addition, hepatocytes were cultured for 24 h in M199 media at 37 °C, and were then pre-incubated with vehicle or 10 μM TPA for 1 h, followed by the addition of 0 μM or 100 μM DHEA for 24 h. After treatment, the protein expression of 17β-HSD and aromatase were determined by western blot.

2.12. AMPK signaling analysis

Primary chicken hepatocytes were cultured in 6-well plates

Table 1
Prime sequence of targeted genes and β-actin.

Gene	GenBank accession number	Primer sequences (5′–3′)	Orientation	Product size(bp)
β-actin	L08165	TGCGTGACATCAAGGAGAAG	Forward	300
		TGCCAGGGTACATTGTGGTA	Reverse	
ACC	J03541	GTTGTGGTTGGCAGAGCAAG	Forward	284
		GCACCAAACTTGAGCACCTG	Reverse	
FAS	NM_205155	TGAAGGACCTTATCGCATTGC	Forward	96
		GCATGGGAAGCATTTTGTTGT	Reverse	
SREBP-1c	AY029224	GTCGGCGATCCTGAGGAA	Forward	105
		CTCTTCTGCACGGCCATCTT	Reverse	
PPARα	AF470455	CAAACCAACCATCCTGACGAT	Forward	64
		GGAGGTCAGCCATTTTTTGGA	Reverse	
CPT-1	AY675193	GGGTTGCCCTTATCGTCACA	Forward	151
		TACAACATGGGCTTCCGTCC	Reverse	
AMPKα1	NM_001039603	CAAGTAGTGTCTCGCACGGT	Forward	133
		GACTGATAGCTGGTCCCACG	Reverse	
AMPKα2	NM_001039605	GTGCACCGAGTCAGAAGTGA	Forward	184
		CGTCCATGAAGGAGCCAGTT	Reverse	
AMPKβ1	NM_001039912	CACCAAGGATGGGGACAGAC	Forward	127
		TTCCAGATCCTGCTGCCAAG	Reverse	
AMPKβ2	NM_001044662	ACACTGCCTTCTTCTCCCTC	Forward	195
		GTGGGAGCTGAAAACACTGG	Reverse	
AMPKγ1	NM_001034827	AGGACTCCTTCAAGCCGTTG	Forward	196
		AACTCAGGCTTTGGGACCTC	Reverse	
AMPKγ2	NM_001278142	ATCACGGTGACACAAGCCCTAC	Forward	179
		GCTATCTGCTTCATTCACTACCACC	Reverse	
AMPKγ3	NM_001031258	CACAAGCGCATCCTCAAGTT	Forward	197
		TGACCGGCAGCATTAACAAC	Reverse	
AR	AB193190	GCCTTCAGTGTCCGTAT CAGCAGCAAGTAGGTGTAT	Forward	333
			Reverse	
ER	X03805	CCCTTCATCCATCACCA	Forward	105
		AGCCCTCACAAGACCAG	Reverse	

(2×10^6 cells per well) for 24 h in M199 at 37 °C. Cells were pre-incubated with vehicle or 10 μM Trilostane for 60 min, followed by addition of 100 μM DHEA. After incubation, the accumulation of lipid droplets was evaluated by Oil Red O staining. Triglyceride content was analyzed using commercial assay kits. Protein expression of AMPK, p-AMPK, ACC, and p-ACC was analyzed by western blot.

Primary chicken hepatocytes were cultured in 6-well plates (2×10^6 cells per well) for 24 h in M199 at 37 °C. One population of cells was pre-incubated with vehicle, 10 μM Flutamide, and 1 μM Fulvestrant for 1 h, followed by addition of 100 μM DHEA for 24 h; another population was treated with 30 nM estradiol, 50 nM testosterone, or 100 μM DHEA for 24 h (estradiol and testosterone concentrations were selected based on our observations of the concentrations of estradiol and testosterone in hepatocytes treated with 100 μM DHEA for 24 h). After incubation, the accumulation of lipid droplets was evaluated by Oil Red O staining assay, and triglyceride content was analyzed using commercial assay kits. In addition, the protein expression of AMPK, p-AMPK, ACC, and p-ACC was analyzed by western blot.

2.13. Data analysis and statistics

Data are expressed as means values ± standard error (SE), and the differences were considered significant at $P < 0.05$. The data which report the time-dependant and dose-dependant effects of DHEA treatment were analyzed by two-way analysis of variance (ANOVA), and followed by a least significant difference *t*-test. The other data were evaluated by one-way ANOVA, followed by post-hoc tests. Treatment differences were subjected to Duncan's multiple comparison test. All statistical analyses were performed with SPSS 20.0 for Windows (StatSoft, Inc., Tulsa, OK, USA).

3. Results

3.1. DHEA treatment improves cell viability, reduces accumulation of lipid droplets, and decreases triglyceride content in primary chicken hepatocytes

Cell viability significantly increased when primary chicken hepatocytes were treated with 0.1–100 μM DHEA for 24 h ($P < 0.01$; Fig. 1A). Biochemical analysis showed that 0.1–100 μM DHEA treatment significantly decreased triglyceride content of primary chicken hepatocytes compared to control treated cells ($P < 0.01$; Fig. 1B). Oil Red O staining was used to analyze the distribution of lipid droplets in cells after DHEA treatment (Fig. 1C). The total area and quantity of lipid droplets were significantly decreased after treatment of primary chicken hepatocytes with 0.1–100 μM DHEA ($P < 0.05$; Fig. 1D and E).

3.2. DHEA modulates expression of lipid metabolism-related factors in primary chicken hepatocytes

The mRNA levels of *ACC*, *FAS*, and *SREBP-1c* were significantly decreased when primary chicken hepatocytes were treated with 0.1–100 μM DHEA ($P < 0.05$; Fig. 2A–C). DHEA treatment (0.1–100 μM) significantly increased *CPT-1* mRNA levels ($P < 0.01$; Fig. 2D), and 50 and 100 μM DHEA treatment significantly increased *PPARα* mRNA levels, compared to control treated cells ($P < 0.05$; Fig. 2E). Compare to the control group, 0.1–100 μM DHEA treatment significantly increased *AMPKα1* and *AMPKα2* mRNA levels ($P < 0.05$; Fig. 2F and G). No differences in *AMPKβ1*, *AMPKβ2*, *AMPKγ1*, *AMPKγ2*, and *AMPKγ3* mRNA levels were observed in primary chicken hepatocytes following DHEA treatment (Fig. 2H–L).

To further validate the impact of DHEA on expression of lipid metabolism-related factors, we examined the protein expression of AMPK and ACC (Fig. 2M). Expression of total AMPK and ACC protein were unchanged in primary chicken hepatocytes after DHEA treatment, while DHEA treatment significantly increased protein expression of p-AMPK and p-ACC ($P < 0.01$; Fig. 2N and O).

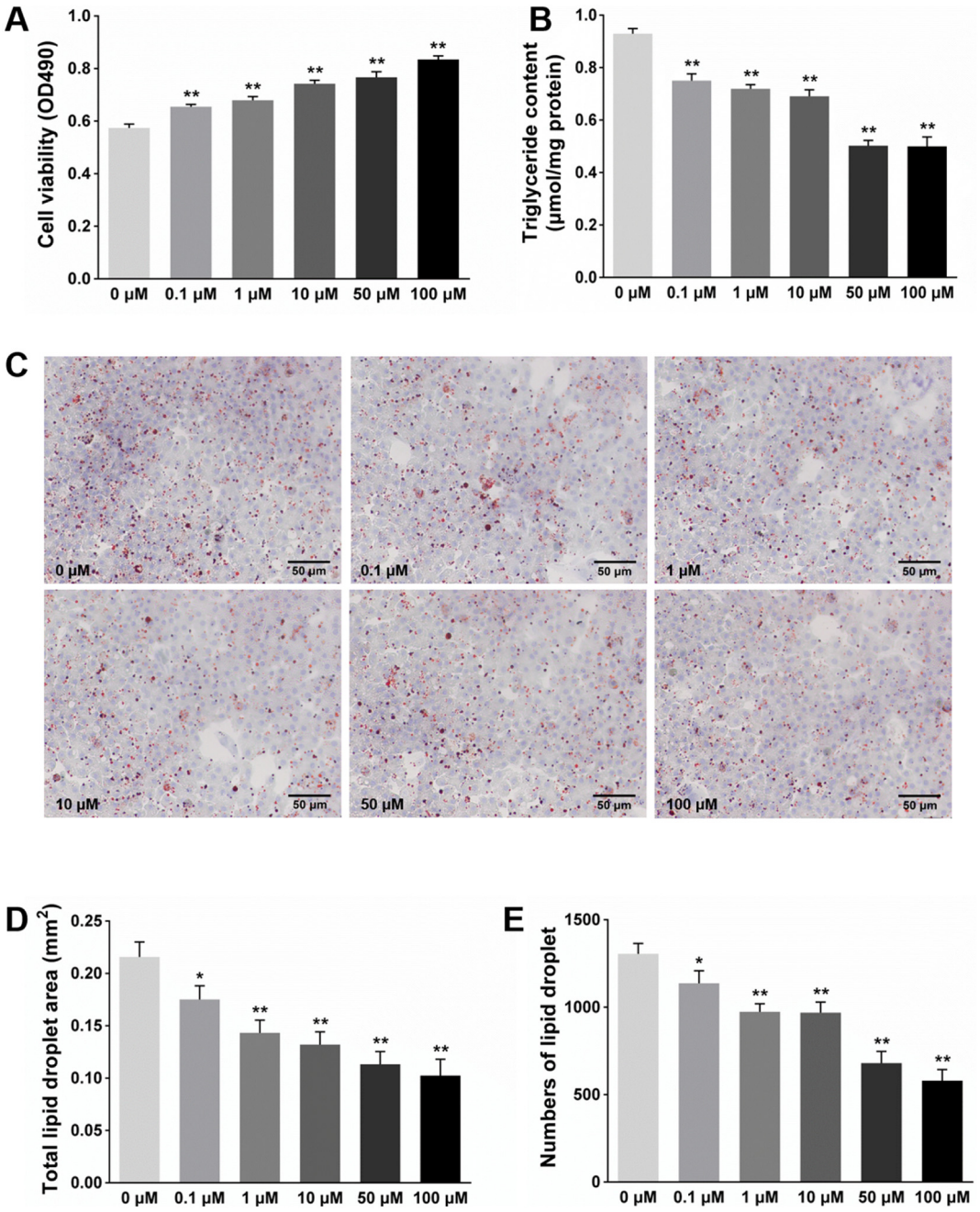

Fig. 1. The effects of DHEA on cell viability and accumulation of lipid droplets in primary chicken hepatocytes.
A: Cell viability; B: Triglyceride content; C: Representative photomicrographs of Oil Red O staining; D: Total area of lipid droplets; E: Quantitation of lipid droplets. Values represent the mean ± SE. $^{**}P < 0.01$ and $^{*}P < 0.05$, compared to control group.

3.3. Biotransformation of DHEA in primary chicken hepatocytes

Testosterone and estradiol were not detected in control treated cells, while testosterone and estradiol contents were significantly increased after DHEA treatment in a dose-dependent manner (Fig. 3A and B). Western blot analysis showed that DHEA treatment significantly increased protein expression of 17β-HSD ($P < 0.01$; Fig. 3C and D). Compared with the control group, 0.1 μM DHEA treatment increased aromatase protein expression ($P < 0.05$), and treatment with 1–100 μM DHEA significantly increased aromatase protein expression ($P < 0.01$; Fig. 3C and E).

3.4. Impact of DHEA on cAMP content and ERK1/2 protein expression in primary chicken hepatocytes

The concentration of cAMP was significantly increased in primary chicken hepatocytes treated with DHEA ($P < 0.05$; Fig. 4A), and remained elevated from 5 min to 60 min in the 100 μM DHEA treatment group (Fig. 4B). The expression of total ERK1/2 protein was unchanged following DHEA treatment, while p-ERK1/2 protein expression was significantly increased in the 0.1–100 μM DHEA treatment groups compared the control group ($P < 0.01$; Fig. 4C). Furthermore, p-ERK1/2 protein expression remained elevated from 10 min to 120 min in the 100 μM DHEA treated group ($P < 0.05$; Fig. 4D).

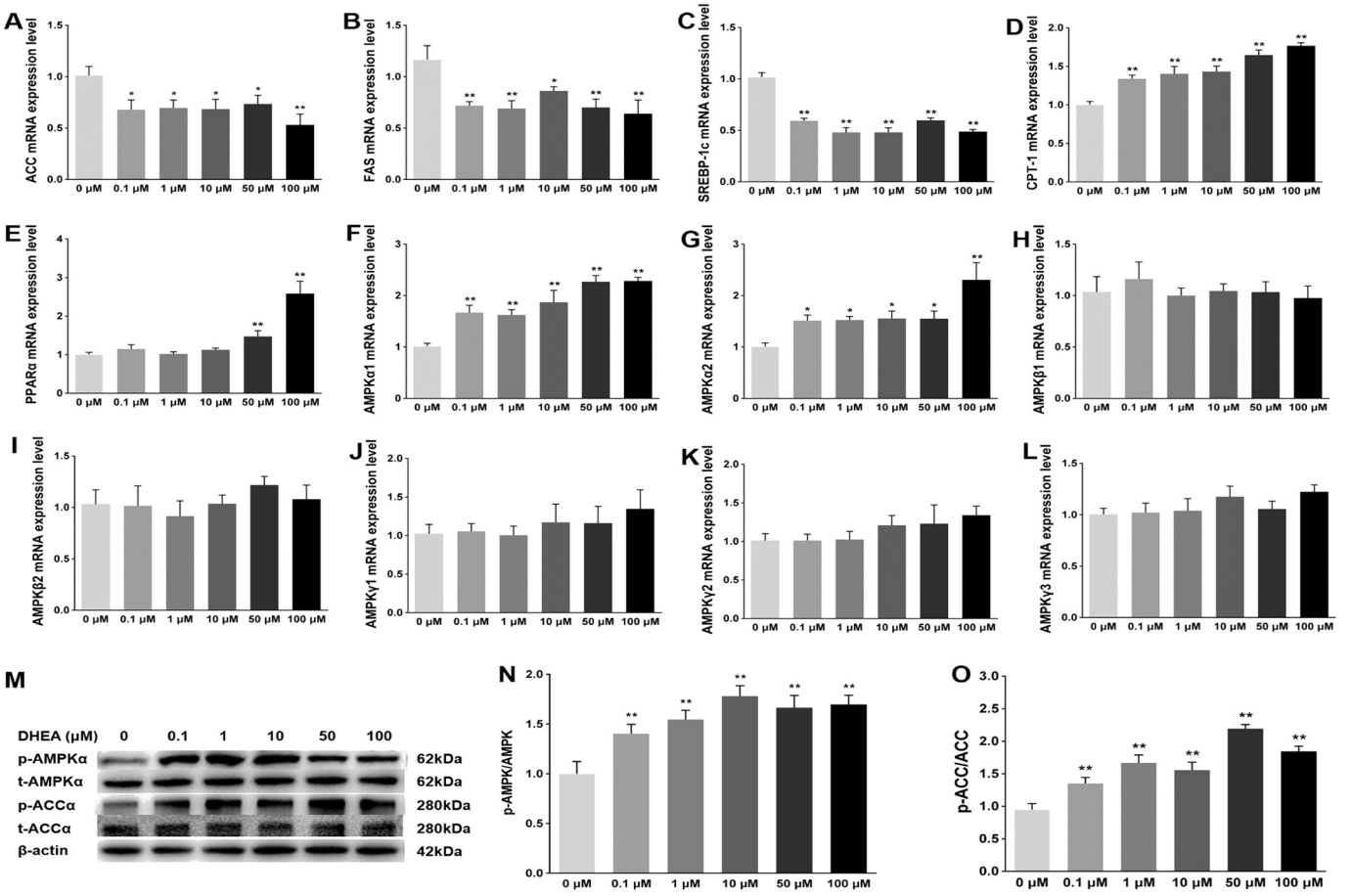

Fig. 2. DHEA modulates expression of lipid metabolism-related genes.
mRNA expression levels of lipid metabolism-related genes following treatment with DHEA: A: Acetyl CoA carboxylase (ACC); B: Fatty acid synthase (FAS); C: Sterol regulatory element binding protein-1c (SREBP-1c); D: Carnitine palmitoyl transferase-1 (CPT-1); E: Peroxisome proliferators-activated receptor α (PPARα); F: Adenosine monophosphate-activated protein kinase α1 (AMPKα1); G: AMPKα2; H: AMPKβ1; I: AMPKβ2; J: AMPKγ1; K: AMPKγ2; L: AMPKγ3; M: Immunoblot of AMPKα, p-AMPKα, ACCα, and p-ACCα protein expression; N: p-AMPK/AMPK; O: p-ACC/ACC. Values are means ± SE. $^{**}P < 0.01$ and $^{*}P < 0.05$, compared to control group.

3.5. cAMP/PKA-ERK1/2 signaling regulates the conversion of DHEA to steroids in primary chicken hepatocytes

Compared to hepatocytes treated with DHEA alone, pre-treatment with H89 (PKA inhibitor) or U0126 (ERK1/2 inhibitor) significantly reduced the increase in testosterone and estradiol content induced by DHEA ($P < 0.05$), while pre-incubation with Forskolin (PKA activator) enhanced the increase of testosterone content induced by DHEA in the 3–48 h treatment groups ($P < 0.05$; Fig. 5A). Similarly, compared to hepatocytes treated with DHEA alone, pre-treatment with H89 or U0126 significantly attenuated the increase in estradiol content induced by DHEA ($P < 0.05$), while pre-incubation with Forskolin enhanced the increase of estradiol content induced by DHEA in the 6–48 h treatment groups ($P < 0.05$; Fig. 5B). Furthermore, the DHEA content in chicken primary hepatocytes was gradually reduced over the duration of treatment; pre-treatment with H89 or U0126 significantly attenuated the decrease of DHEA content ($P < 0.05$) in the 6–48 h treatment groups, while pre-incubation with Forskolin enhanced the decrease of DHEA content in the 6–48 h treatment groups ($P < 0.05$; Fig. 5C). These results indicate that cAMP/PKA-ERK1/2 signaling plays an important role in the conversion of DHEA to active steroid metabolites.

DHEA treatment did not induce changes in the total protein abundance of ERK1/2, while DHEA treatment did significantly increase p-ERK1/2 protein expression, compared to the control group ($P < 0.01$; Fig. 5D and F). The activation of p-ERK1/2 by DHEA was significantly inhibited by pre-treatment with H89 or U0126, while activation of p-ERK1/2 was enhanced by pre-incubation with Forskolin ($P < 0.01$; Fig. 5D and F). These results suggest that DHEA acts through PKA to regulate the activation of p-ERK1/2.

To further investigate whether the changes in 17β-HSD and aromatase protein expression induced by DHEA are dependent on activation of the cAMP/PKA-/ERK1/2 signaling pathway, the hepatocytes were pre-treated with H89 (PKA inhibitor), U0126 (ERK1/2 inhibitor), TPA (ERK1/2 activator), or Forskolin (PKA activator) before addition of DHEA treatment. The increases in 17β-HSD and aromatase protein expression induced by DHEA were significantly inhibited when hepatocytes were pre-treated with H89 or U0126 ($P < 0.01$), while pre-incubation of hepatocytes with Forskolin or TPA strongly enhanced the protein expression of 17β-HSD and aromatase ($P < 0.01$; Fig. 5E, G–K). These results suggested that DHEA induces increases in 17β-HSD and aromatase protein expression through activation of the cAMP/PKA-ERK1/2 signaling pathway in primary chicken hepatocytes.

3.6. Impact of DHEA on steroid hormone receptor expression in primary chicken hepatocytes

DHEA treatment significantly increased *ER* mRNA levels in primary chicken hepatocytes ($P < 0.01$; Fig. 6A), while no changes were observed in *AR* mRNA levels treated with DHEA ($P > 0.05$; Fig. 6B). To further certify these results, the content of ER and AR protein was detected using ELISA kits; these assays also demonstrated no change in AR protein content due to DHEA treatment (Fig. 6E), while treatment with 0.1–100 μM DHEA significantly increased ER protein content in

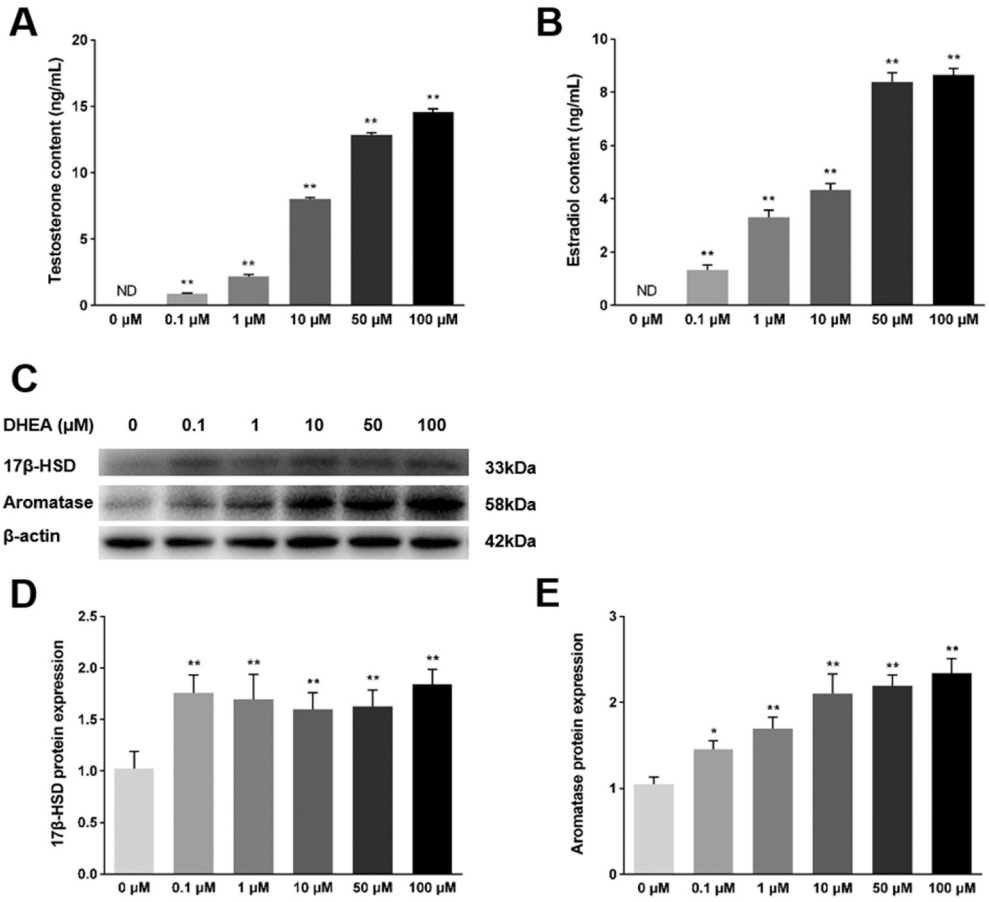

Fig. 3. Biotransformation of DHEA to active steroid hormones in primary chicken hepatocytes.
A: Testosterone content; B: Estradiol content; C: Immunoblot of 17β-HSD and aromatase protein expression; D: 17β-HSD protein expression; E: aromatase protein expression. Values are means ± SE. $^{**}P < 0.01$ and $^{*}P < 0.05$, compared to control group.

primary chicken hepatocytes ($P < 0.05$; Fig. 6C). ER protein content was also significantly increased in primary chicken hepatocytes treated with 100 μM DHEA for 12–48 h ($P < 0.05$; Fig. 6D), while no changes were observed in AR protein content ($P > 0.05$; Fig. 6F).

3.7. Conversion of DHEA to estradiol modulates lipid metabolism in primary chicken hepatocytes

Pre-treatment with Trilostane (an inhibitor of 3β-HSD) can inhibit the conversion of DHEA to active downstream hormones, including testosterone and estradiol. DHEA treatment significantly decreased the triglyceride content, and the quantity and total area of lipid droplets ($P < 0.01$); this effect was significantly reversed in primary chicken hepatocytes pre-treated with Trilostane ($P < 0.01$; Fig. 7A–D). Similarly, the increase in p-AMPK and p-ACC protein expression induced by DHEA was also significantly reversed by pre-incubation with Trilostane ($P < 0.01$; Fig. 7E–G). These results indicate that the mechanism of action DHEA by which treatment reduces lipid droplet accumulation in primary chicken hepatocytes is mediated indirectly by the conversion of DHEA into active steroid hormones, not through direct action of DHEA itself.

Treatment with DHEA or estradiol significantly decreased triglyceride content and reduced the quantity and total area of lipid droplets in primary chicken hepatocytes ($P < 0.01$), while no changes were observed in triglyceride content or in the quantity and total area of lipid droplets in hepatocytes treated with testosterone alone ($P > 0.05$; Fig. 8A–D). In addition, compared with the DHEA treatment group, pre-treatment with Fulvestrant (ER antagonist) significantly reversed the decrease of triglyceride content and lipid droplet accumulation induced by DHEA ($P < 0.01$); the decrease of triglyceride content and droplets accumulation induced by DHEA was unaffected in hepatocytes pre-treated with Flutamide (AR antagonist) ($P > 0.05$; Fig. 8A–D).

The protein expression of p-AMPK and p-ACC were significantly increased in hepatocytes after treatment with DHEA or estradiol ($P < 0.01$), whereas no changes were observed in hepatocytes following testosterone treatment alone ($P > 0.05$; Fig. 8E–G). Similarly, no effect was observed on protein expression of p-AMPK and p-ACC in hepatocytes pre-treated with Flutamide ($P > 0.05$), while the increase of p-AMPK and p-ACC protein expression induced by DHEA or estradiol alone was significantly reversed in hepatocytes pre-treated with Fulvestrant ($P < 0.01$; Fig. 8E–G). These results suggest that DHEA regulates lipid metabolism in primary chicken hepatocytes primarily through conversion into estrogens, likely primarily through estradiol.

4. Discussion

Dehydroepiandrosterone (DHEA), a naturally occurring steroid, is produced by the adrenal cortex in an age-dependent pattern [35], and the age-related decline in DHEA production has attracted interest as a public health issue [36]. The enzymes largely responsible for the biotransformation of DHEA into androgens and estrogens are in peripheral target tissues in which steroid hormone synthesis occurs, and locally produced steroid hormones exert their actions in cells without significant diffusion into the circulation, thus seriously limiting the interpretation of serum levels of active steroid hormones [37]. DHEA is only converted into active steroid hormone metabolites in the specific target tissues where the appropriate enzymatic machinery exists, thus limiting the adverse effects of systemic hormone treatment with DHEA

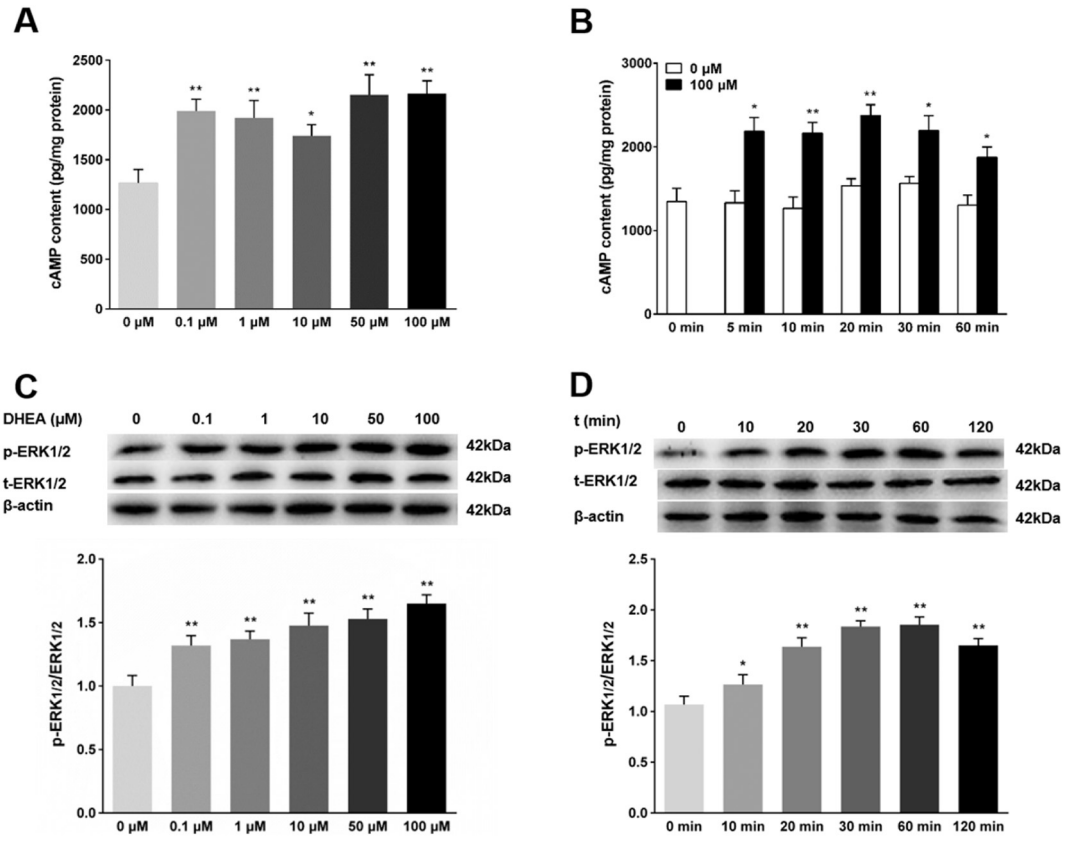

Fig. 4. The effect of DHEA on cAMP content and ERK1/2 protein expression in primary chicken hepatocytes.
A: Cyclic 3′, 5′-adenosine monophosphate (cAMP) content in primary chicken hepatocytes treated with different dosages of DHEA; B: The cAMP content in hepatocytes treated with 100 μM DHEA for various time; C: The p-ERK1/2 protein expression in hepatocytes treated with different doses of DHEA for 24 h; D: The p-ERK1/2 protein expression in hepatocytes treated with 100 μM DHEA for various times. Values represent the mean ± SE. $^{**}P < 0.01$ and $^{*}P < 0.05$, compared to control group.

compared to estradiol or testosterone [9]. Currently, DHEA is commercially available as a non-prescription nutritional supplement [38].

Many studies have reported that administration of DHEA reduces weight gain and accumulation visceral fat in animals [18,39] and humans [36,40]. In previous studies, we reported that DHEA reduced fat accumulation in broiler chickens and during embryonic development [19,20]. Assessment of lipid droplet accumulation is important in the evaluation of the overall lipid metabolic status of a tissue or organism [41]. In the present study, we demonstrate that the quantity and total area of lipid droplets in cultured primary chicken hepatocytes are significantly decreased following treatment with DHEA. Consistent with these results, DHEA treatment also significantly decreased the triglyceride content in primary chicken hepatocytes. These results indicate that DHEA treatment suppresses lipid droplet accumulation in primary chicken hepatocytes.

It was reported that DHEA reduces fat deposition by activating the cAMP-PKA signaling pathway [30], and inducing the expression of relevant metabolic genes [30]. The AMPK signaling pathway plays an important role in the regulation of energy metabolism in the liver. However, little is known regarding whether DHEA regulates lipid metabolism by activing AMPK signaling in poultry. The AMPK signaling pathway regulates expression of lipid metabolism transcription factors, such as ACC, PPARα, and SREBP-1c [27]. Our data demonstrate that DHEA treatment significantly increases *AMPKα* mRNA levels, but not levels of *AMPKβ* or *AMPK*γ in primary chicken hepatocytes. We also report that DHEA treatment significantly increases p-AMPK protein expression in primary chicken hepatocytes. This result is similar to one study, in which DHEA was shown to regulate lipid metabolism in obese rats by activating the AMPK signaling pathway [18]. DHEA was also reported to reduce intracellular energy by activating AMPKα2 in rat cardiac myocytes [28]. Activated AMPK turns on the lipid catabolism pathway and shuts down lipid biosynthesis [42]. Some key enzymes of lipid metabolism, such as FAS and ACC, are downstream factors of p-AMPK [43]. Our results demonstrate that the *ACC* and *FAS* mRNA levels are significantly decreased in primary chicken hepatocytes after DHEA treatment. These results are consistent with previous reports that DHEA accelerates lipid anabolism by regulating hepatic gene expression in primary chicken hepatocytes [30]. Additionally, we found that DHEA treatment significantly decreases *SREBP-1c* mRNA levels in primary chicken hepatocytes. SREBP serves as a regulator of biosynthesis of cholesterol and fatty acids in the liver [44]. SREBP-1c is potentially a regulator of lipid metabolism, and can directly stimulate the transcription of genes encoding FAS enzymes [45]. Fat accumulation is a complex process involving the balance of lipogenesis and lipolysis. PPARα influences lipid metabolism by regulating the expression of genes involved in fatty acid β-oxidation, such as CPT-1 [46]. In the present study, we report that DHEA treatment significantly increases *PPARα* and *CPT-1* mRNA levels in primary chicken hepatocytes. It is hypothesized that DHEA increases fat oxidation by inhibiting malonyl-CoA formation, which would activate carnitine palmitoyl transferase-I (CPT-1) activity [47]. Considering the reduction of lipid droplet accumulation and triglyceride content, it is possible that DHEA inhibits lipogenesis by inhibiting expression of SREBP-1c, ACC, and FAS; likewise DHEA may accelerate lipolysis by enhancing expression of PPARα and CPT-1, resulting in reduced fat deposition in primary chicken hepatocytes. Thus, these results indicate that the effects of DHEA treatment on expression of lipid metabolism-related factors in primary chicken hepatocytes were mainly achieved by enhancing phosphorylation of the AMPK protein.

The fat-reducing effects of DHEA likely involve multiple

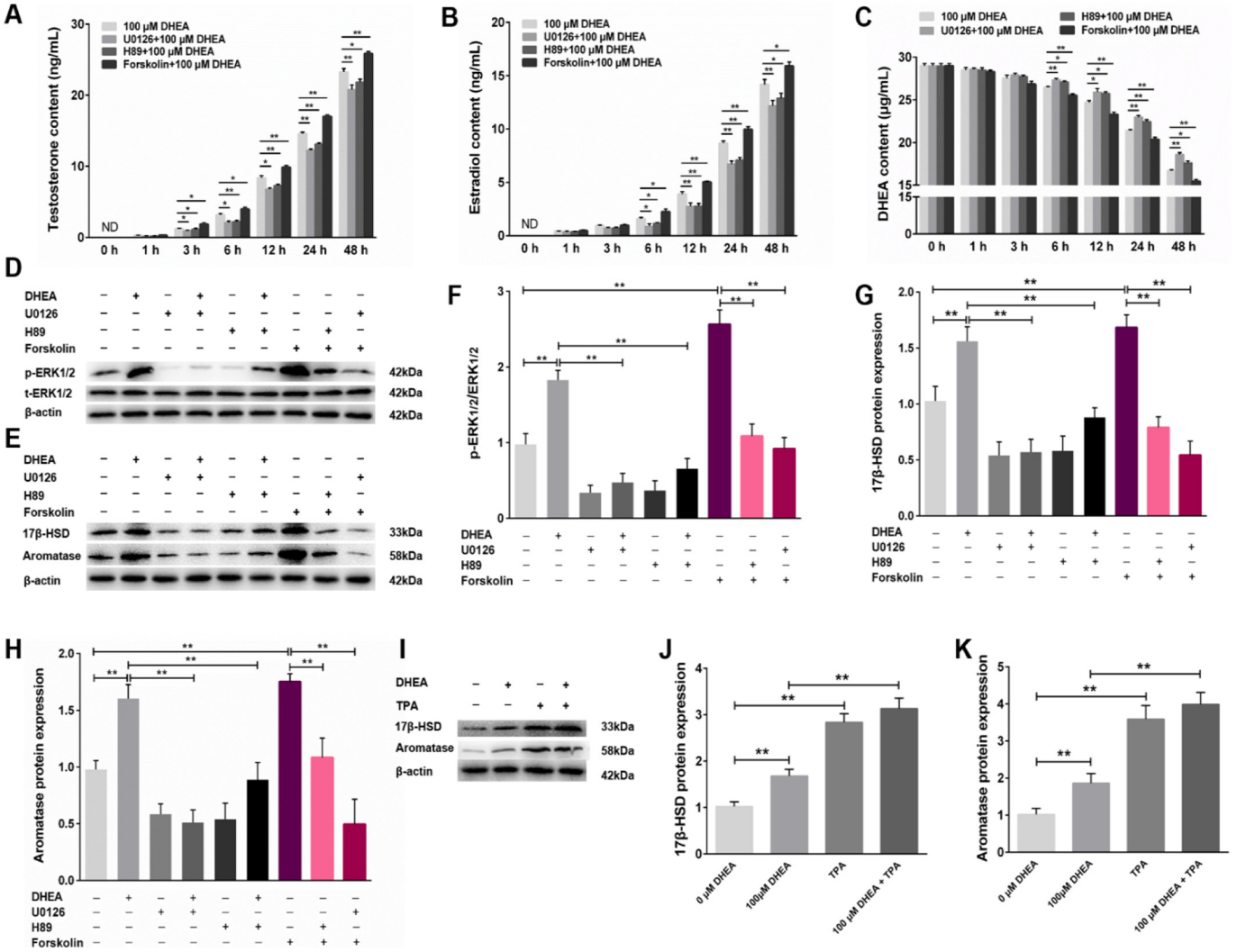

Fig. 5. The role of the cAMP/PKA-ERK1/2 signaling on the conversion of DHEA to active steroid hormones in primary chicken hepatocytes.
Hepatocytes were cultured in serum free medium for 24 h and pre-treated with 20 μM Forskolin, 10 μM H89, or 10 μM U0126 for 1 h, followed by the addition of 100 μM DHEA for varying lengths of time. A: Testosterone content; B: Estradiol content; C: DHEA content; D: Immunoblot of ERK1/2 and p-ERK1/2 expression in hepatocytes treated with DHEA, U0126, H89, or Forkolin; E: Immunoblot of 17β-HSD and aromatase expression in hepatocytes treated with DHEA, U0126, H89, or Forkolin; F: p-ERK1/2/ERK1/2 protein expression; G: 17β-HSD protein expression; H: Aromatase protein expression; I: Immunoblot of 17β-HSD and aromatase expression in hepatocytes treated with DHEA or TPA; J: 7β-HSD protein expression; K: Aromatase protein expression. Values represent mean ± SE. $^{**}P < 0.01$ and $^{*}P < 0.05$, compared to the respective control group.

mechanisms [48]. DHEA acts either indirectly by conversion to androgens or estrogens in peripheral target tissues, or directly by interacting with neurotransmitter receptors in the brain as a neurosteroid [49]. It has been reported that the putative protective role of DHEA in obesity is related to its conversion to active hormones [50,51]. Since it both estrogen and androgens can influence lipid metabolism, we hypothesized that DHEA modulates lipid metabolism *via* peripheral conversion to estrogens or androgens in primary chicken hepatocytes. In our study, the contents of testosterone and estradiol were increased in a dose-dependent manner in primary chicken hepatocytes treated with DHEA. These results are consistent with other reports that treatment with DHEA can enhance testosterone and estradiol content *in vivo* and *in vitro* [52,53]. The steroidogenic enzymes expression level and steroid hormone content are generally positively correlated. Androgens upregulate aromatase expression level in adult rat germ cells, whereas estrogens have opposite effect [54]. Li et al. reported that DHEA can be converted into estradiol by enhancing aromatase activity [55]. Our present results demonstrate that DHEA treatment significantly increases protein abundance of 17β-HSD and aromatase in primary chicken hepatocytes. In peripheral target tissues, DHEA is converted into androstenedione by 3β-HSD, and then is converted to testosterone or estradiol by 17β-HSD or aromatase, respectively [12]. Although we did not detect the protein expression of 3β-HSD, the increase of 17β-HSD and aromatase protein abundance induced by DHEA indicates that DHEA treatment increases expression of steroidogenic enzymes that in turn may enhance the conversion of DHEA into estradiol and testosterone in primary chicken hepatocytes.

The ERK1/2 signaling pathway is thought to play a critical role in steroidogenesis [15]. After stimulation by extracellular signals, activated ERKs regulate signaling kinases that participate in the control of steroidogenesis in granulosa-derived cell lines [56]. We previously reported that DHEA regulates testosterone biosynthesis by activating the ERK1/2 signaling pathway in primary rat Leydig cells [17]. In this study, we found no difference in total ERK1/2 protein expression after DHEA treatment, while the expression of p-ERK1/2 protein significantly increased in primary chicken hepatocytes treated with different doses of DHEA, which indicates that DHEA treatment cab activate ERK1/2 signaling in primary chicken hepatocytes. In fact, crosstalk between cAMP/PKA and ERK1/2 pathways has been previously described in the human adrenal NCI-H295R cell line [57]. In this study, we found that treatment with a wide range of DHEA concentrations enhanced cAMP content in primary chicken hepatocytes; the maximal

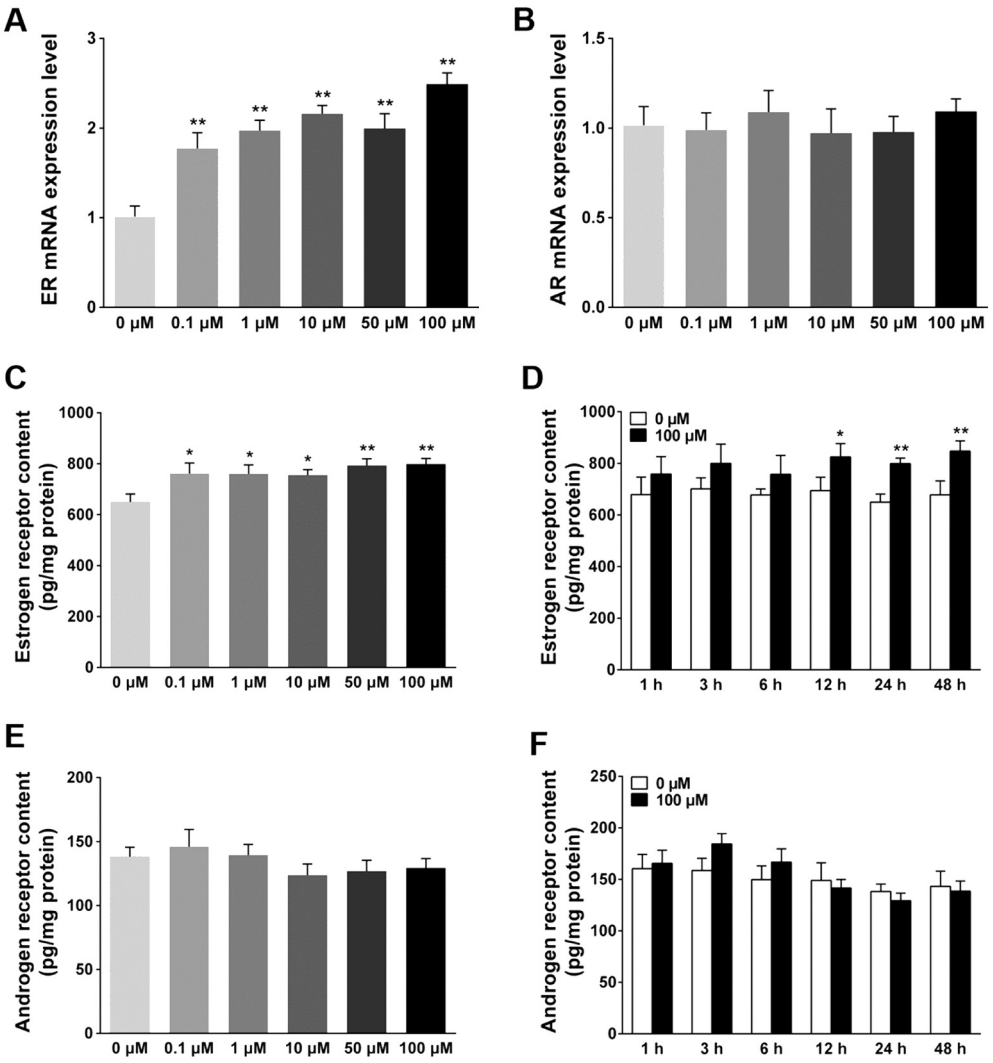

Fig. 6. Effect of DHEA on steroid hormone receptor in primary chicken hepatocytes.
A: Estrogen receptor (ER) mRNA level; B: Androgen receptor (AR) mRNA level; C: ER protein content in hepatocytes treated with different doses of DHEA for 24 h; D: ER protein content in hepatocytes treated with 100 μM DHEA for various times; E: AR protein content in hepatocytes treated with different doses of DHEA for 24 h; F: AR protein content in hepatocytes treated with 100 μM DHEA for various times. Values are means ± SE. $^{**}P < 0.01$ and $^{*}P < 0.05$, compared to control group.

increase follows treatment with 100 μM DHEA. Additionally, the cAMP content remained elevated from 5 min to 60 min in primary chicken hepatocytes treated with 100 μM DHEA. In general, the half-life of cAMP is 30 min, and it is in dynamic equilibrium. Therefore, the increase in intracellular cAMP content is often transient, initially showing an excessive increase, and then is reduced by feedback inhibition mechanisms of the signaling pathway. These results suggest that DHEA treatment increases cAMP accumulation in primary chicken hepatocytes; this is consist with our previous report that DHEA accelerates lipid catabolism by activating the cAMP/PKA signaling pathway in primary chicken hepatocytes [30]. Thus, we speculated that the cAMP/PKA-ERK1/2 signaling pathway may play an important role in the conversion of DHEA to active steroid hormones in primary chicken hepatocytes. Importantly, the present study found that pre-treated with Forskolin significantly increased the content of testosterone and estradiol in primary chicken hepatocytes induced by DHEA; the increase of testosterone and estradiol induced by DHEA was significantly reversed in hepatocytes pre-treated with H89 or U0126. In addition, we also found that pre-treatment with Forskolin significantly enhanced the reduction of DHEA content in primary chicken hepatocytes, while pre-treatment with H89 or U0126 significantly blocked the decrease of DHEA content. These results indicate that cAMP/PKA signaling plays an important role in the conversion of DHEA to testosterone and estradiol. Interestingly, the activation of p-ERK1/2 induced by DHEA was significantly inhibited by pre-treatment with H89 or U0126, while activation of p-ERK1/2 was enhanced by pre-incubation with Forskolin (PKA activator). These results suggest that DHEA regulates the activation of p-ERK1/2 through PKA. Consistent with the changes observed in active steroid hormone and DHEA contents, and in p-ERK1/2 protein expression, we found that the 17β-HSD and aromatase protein expression induced by DHEA was significantly reversed in primary chicken hepatocytes pre-treated with H89 or U0126, while pre-treatment with Forskolin or TPA significant enhanced the increase in 17β-HSD and aromatase protein expression induced by DHEA; this suggests that DHEA induces increased expression of 17β-HSD and aromatase protein by activation of cAMP/PKA-ERK1/2 signaling in primary chicken hepatocytes. Therefore, we speculated that DHEA treatment accelerates the conversion of DHEA into testosterone and estradiol by enhancing the protein expression of 17β-HSD and aromatase, which is achieved by activation of the cAMP/PKA-ERK1/2 signaling pathway in primary chicken hepatocytes.

Our results indicate that DHEA is converted into active steroid hormone and regulates lipid metabolism in primary chicken hepatocytes. To find out whether DHEA directly or indirectly exerts its

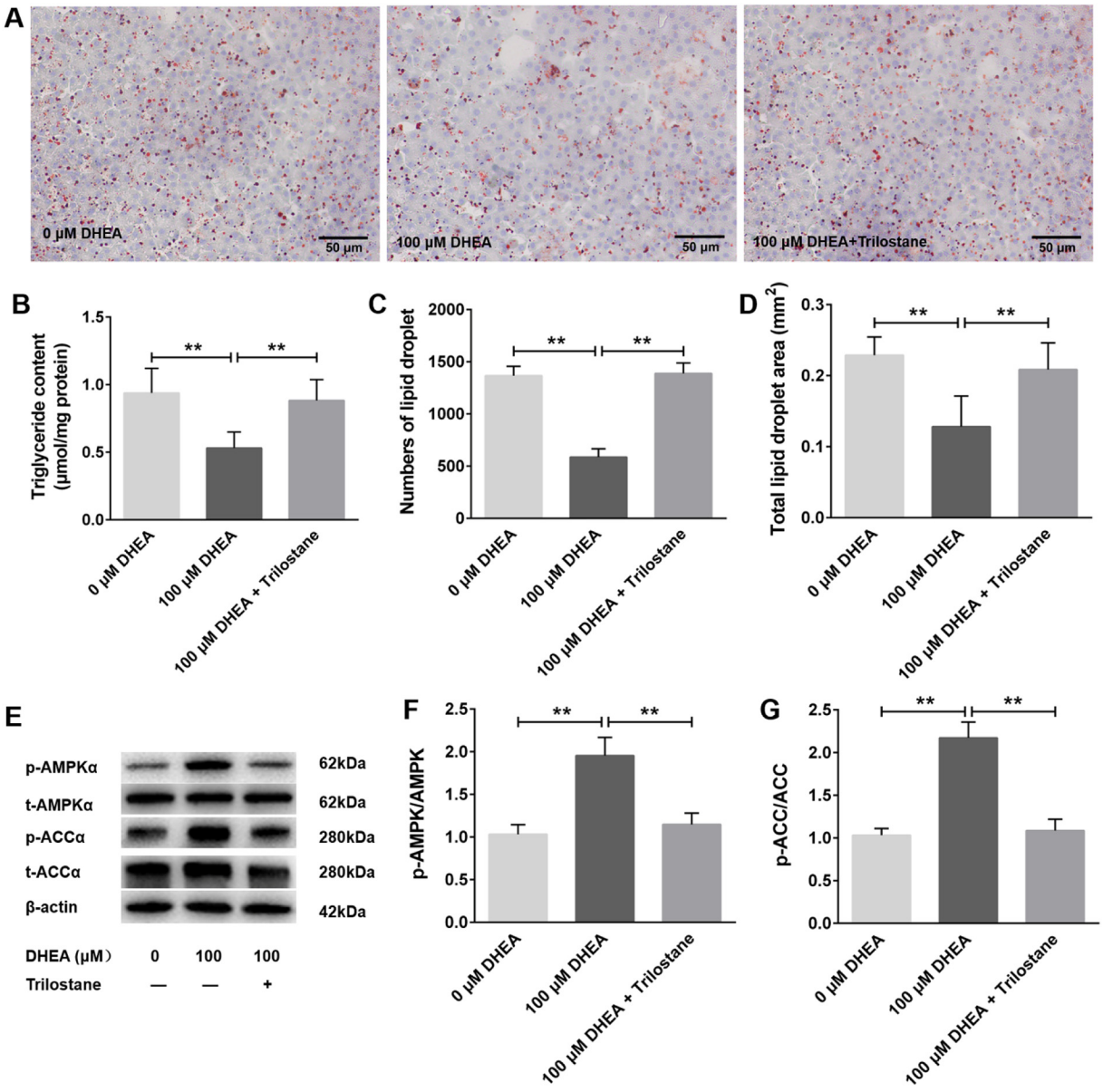

Fig. 7. DHEA reduces accumulation of lipid droplet by conversion into active steroid hormones in primary chicken hepatocytes.
Hepatocytes were pre-treated with vehicle or 10 μM Trilostane for 1 h, followed by the addition of 100 μM DHEA for 24 h. Triglyceride content was determined using commercial kits; samples were stained with Oil Red O and photomicrographs were taken to determine the accumulation of lipid droplets, and protein expression of AMPKα, p-AMPKα, ACCα, and p-ACCαwas determined by western blot. A: Representative photomicrographs of Oil Red O staining; B: Evaluation of triglyceride content; C: Quantitation of lipid droplet accumulation; D: Total are of lipid droplets; E: Immunoblot of AMPKα, p-AMPKα, ACCα, and p-ACCα expression; F: p-AMPK/AMPK protein expression; G: p-ACC/ACC protein expression. Values represent mean ± SE. $^{**}P < 0.01$ and $^{*}P < 0.05$, compared to the respective control group.

function on lipid metabolism in primary chicken hepatocytes, cells were pre-treated with Trilostane, an inhibitor of 3β-HSD, the first key enzyme of DHEA biotransformation. We report that DHEA treatment significantly decreases the triglyceride content and the quantity and total area of lipid droplets in primary chicken hepatocytes; this effect was blocked by pre-treatment with Trilostane. Similarly, we found that the increase of p-AMPK and p-ACC protein expression induced by DHEA was also blocked by pre-treatment with Trilostane. These data suggest that the effects of DHEA on lipid metabolism are mediated indirectly through the conversion of DHEA into active steroid hormones in chicken hepatocytes, not through direct action of DHEA itself. Intriguingly, we found that DHEA treatment significantly increased expression of estrogen receptor mRNA levels and protein content in primary chicken hepatocytes, whereas no differences were observed in expression of androgen receptor mRNA levels or protein content. These results suggest that the fat-reducing effects of DHEA are mediated through its conversion into estradiol in primary chicken hepatocytes. Previous studies reported that estradiol can regulate lipid metabolism and prevent obesity and diabetes in humans [22,23]. To further determine which metabolites of DHEA are the primary factors influencing the reduction of lipid droplet accumulation in hepatocytes, we evaluated the accumulation of lipid droplets in primary chicken hepatocytes treated with DHEA, testosterone, estradiol, Fulvestrant, or Flutamide. In addition, in order to ensure the biological relevance of our results, the dosages of testosterone and estradiol were based on our observations of the concentration of estradiol and testosterone in hepatocytes treated with 100 μM DHEA for 24 h. After DHEA or estradiol treatment, the triglyceride content and accumulation of lipid droplets were significantly decreased, while the p-AMPK and p-ACC protein expression were significantly increased; these actions of DHEA and estradiol were blocked by pretreatment with Fulvestrant. In contrast, no differences were observed in the triglyceride content, lipid droplet accumulation,

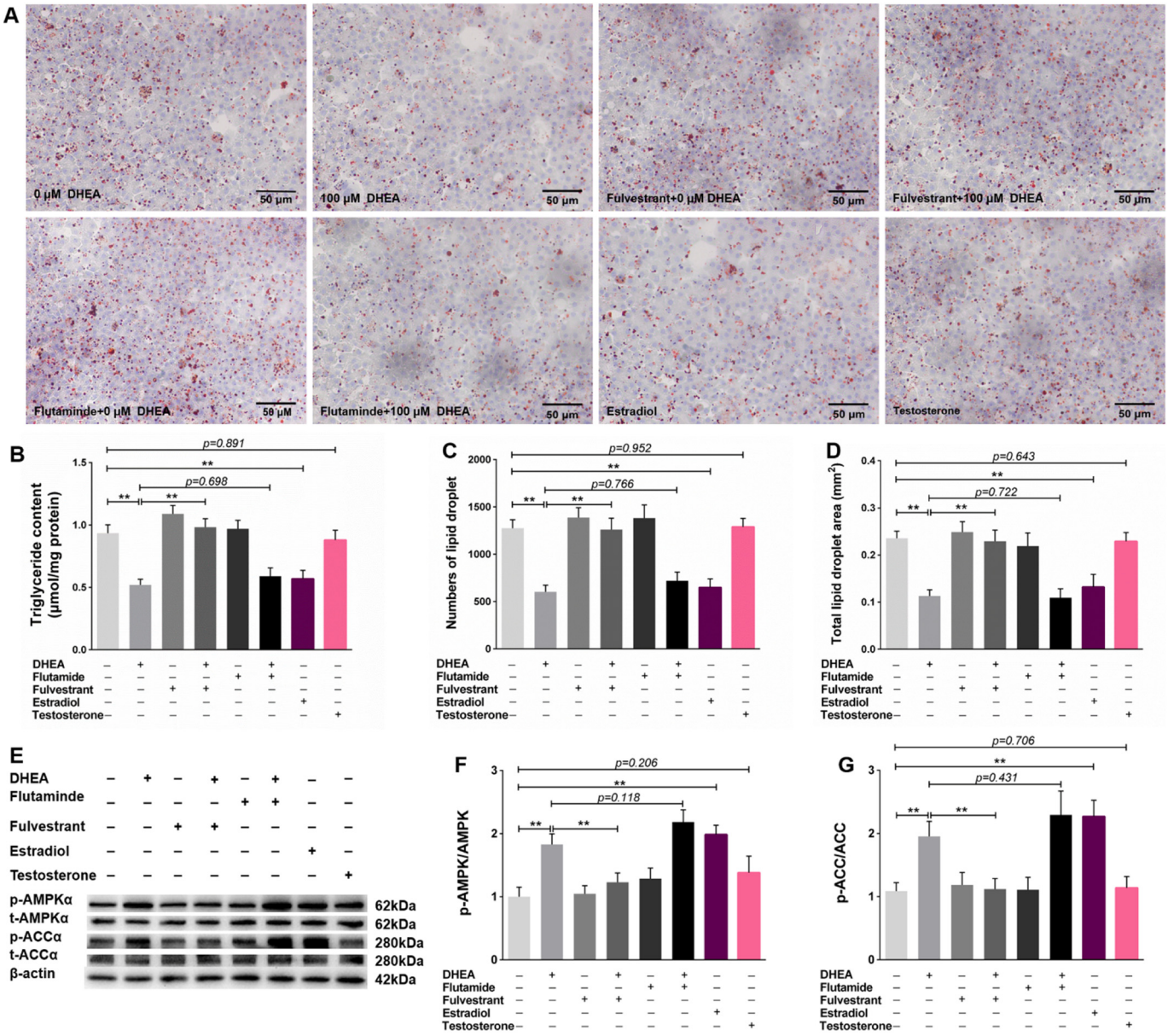

Fig. 8. DHEA modulates lipid metabolism through conversion into estradiol in primary chicken hepatocytes.
Hepatocytes were treated with 30 nM estradiol or 50 nM testosterone, or were pre-incubated with vehicle, 10 μM Flutamide and 1 μM Fulvestrant for 1 h, then followed by the addition of 100 μM DHEA for 24 h. Triglyceride content was determined using commercial kits. Samples were stained with Oil Red O and photomicrographs were taken to evaluate the accumulation of lipid droplets, and protein expression of AMPKα, p-AMPKα, ACCα, and p-ACCα were determined by western blot. A: Representative photomicrographs of Oil Red O staining; B: Triglyceride content; C: Quantitation of lipid droplets; D: Total area of lipid droplets; E: Immunoblot of AMPKα, p-AMPKα, ACCα, and p-ACCα protein expression; F: Relative p-AMPK protein expression; G: Relative p-ACC protein expression. Values represent mean ± SE. $^{**}P < 0.01$ and $^{*}P < 0.05$, compared to the respective control group.

or the p-AMPK and p-ACC protein expression in primary chicken hepatocytes treated with testosterone or pre-treated with Flutamide. Based on these results, we suggest that DHEA regulates lipid metabolism in primary chicken hepatocytes primarily through conversion into estrogens, primarily estradiol. Thus, we speculate that the activations of p-AMPK and p-ACC induced by DHEA that subsequently reduced lipid droplet accumulation was mediated indirectly through the conversion of DHEA into estradiol in chicken hepatocytes, not through direct action of DHEA itself.

In conclusion, our data demonstrate that DHEA represses lipogenesis by inhibiting expression of SREBP-1c, ACC, and FAS, and accelerates lipolysis by enhancing PPARα and CPT-1 expression levels; this is ultimately reduced the accumulation of lipid droplets in primary chicken hepatocytes. DHEA exerts its fat-reducing effects *via* biotransformation into active steroid hormones, and this is mainly associated with enhancement of p-AMPK protein expression in primary chicken hepatocytes. Additionally, we show that DHEA increases expression of 17β-HSD and aromatase protein by activating the cAMP/PKA-ERK1/2 signaling cascade, which ultimately leads to the conversion of DHEA into testosterone and estradiol in primary chicken hepatocytes. Importantly, we demonstrate that the effect of DHEA on lipid metabolism-related factors is mainly mediated by conversion of DHEA into estradiol, not testosterone, and not through direct action of DHEA itself. The results presented here further elucidate the molecular mechanisms underlying the fat-reducing action of DHEA, and further suggest the potential for use of DHEA to control fat deposition in the treatment of lipid metabolism-related diseases.

Conflict of interest statement

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled, “Dehydroepiandrosterone reduces accumulation of lipid droplets in primary chicken hepatocytes by biotransformation mediated *via* the cAMP/PKA-ERK1/2 signaling pathway”.

Transparency document

The Transparency document associated with this article can be found, in online version.

Acknowledgments

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Competing interests

The authors declare that they have no competing interests.

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