The regulation of stearoyl-CoA desaturase gene expression is tissue specific in chickens

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Abstract

Emerging evidence suggests a potential role of stearoyl-CoA desaturase (SCD)-1 in the control of body weight and energy homeostasis. The present study was conducted to investigate the effects of several energy balance-related factors (leptin, cerulenin, food deprivation, genotype, and gender) on SCD gene expression in chickens. In experiment 1, 6-week-old female and male broiler chickens were used. In experiment 2, two groups of 3-week-old broiler chickens were continuously infused with recombinant chicken leptin (8 µg/kg/h) or vehicle for 6 h. In experiment 3, two groups of 2-week-old broiler chickens received i.v. injections of cerulenin (15 mg/kg) or vehicle. In experiment 4, two broiler chicken lines (fat and lean) were submitted to two nutritional states (food deprivation for 16 or 24 h and feeding *ad libitum*). At the end of each experiment, tissues were collected for analyzing SCD gene expression. Data from experiment 1 showed that SCD is ubiquitously expressed in chicken tissues with highest levels in the proventriculus followed by the ovary,

hypothalamus, kidney, liver, and adipose tissue in female, and hypothalamus, leg muscle, pancreas, liver, and adipose tissue in male. Female chickens exhibited significantly higher SCD mRNA levels in kidney, breast muscle, proventriculus, and intestine than male chickens. However, hypothalamic SCD gene expression was higher in male than in female ($P < 0.05$). Leptin increased SCD gene expression in chicken liver ($P < 0.05$), whereas cerulenin decreased SCD mRNA levels in muscle. Both leptin and cerulenin significantly reduced food intake ($P < 0.05$). Food deprivation for either 16 or 24 h decreased the hepatic SCD gene expression in fat line and lean line chickens compared with their fed counterparts ($P < 0.05$). The hypothalamic SCD mRNA levels were decreased in both lines only after 24 h of food deprivation ($P < 0.05$). In conclusion, SCD is ubiquitously expressed in chickens and it is regulated by leptin, cerulenin, nutritional state, and gender in a tissue-specific manner.

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Introduction

Stearoyl-CoA desaturase (SCD) is an integral membrane protein of the endoplasmic reticulum that catalyzes the rate-limiting step in the biosynthesis of monounsaturated fatty acids from saturated fatty acids (Heineman & Ozols 2003, Miyazaki & Ntambi 2003). SCD induces, in conjunction with NADPH-cytochrome *b5* reductase and cytochrome *b5*, a *cis*-configuration double bond into its substrates, palmitic and stearic acid, to generate the products, palmitoleic and oleic acid (Enoch *et al.* 1976). These monounsaturated fatty acids are used as substrates for the synthesis of triglycerides, wax esters, cholesteryl esters, and membrane phospholipids.

Four SCD isoforms have been characterized in the mice (Ntambi *et al.* 1988, Kaestner *et al.* 1989, Zheng *et al.* 2001, Miyazaki *et al.* 2003). These isoforms displayed similar desaturation activities towards stearoyl-CoA and palmitoyl-CoA, but have different tissue distributions. Mouse SCD-1 (mSCD-1) is expressed in a broad range of tissues and at high

levels in liver, adipose tissue, preputial gland, and Harderian gland. Highest mSCD-2 expression was detected in brain and Harderian gland, while mSCD-3 expression is limited to Harderian gland. Mouse SCD-4 appears to be heart specific. Two SCD isoforms have been identified in human (hSCD-1 and -5; Zhang *et al.* 1999, Wang *et al.* 2005) and in rat (rSCD-1 and -2; Mihara 1990). Human SCD-5 was abundantly expressed in brain and pancreas (Wang *et al.* 2005). In birds, one SCD isoform (SCD) has been, so far, identified and cloned (GenBank accession number X60465) and very little is known about its tissue distributions and regulation (Lefevre *et al.* 1999, 2001).

Increasing evidence suggests that SCD-1 plays a crucial role in lipid metabolism and body weight control in mammals. Indeed, asebia mice (homozygous for a naturally occurring mutation that results in the lack of SCD-1 expression; Zheng *et al.* 1999) and SCD-1 knockout mice are lean and hypermetabolic (Ntambi *et al.* 2002). Recently, SCD-1 was found to be a major peripheral target of leptin (Cohen *et al.* 2002), a key regulator of energy homeostasis and satiety.

Leptin reduces liver SCD-1 mRNA expression as well as its enzymatic activity, which contributes to food intake reduction and weight loss in mice (Cohen *et al.* 2002). ob/ob mice with SCD-1 mutations were significantly less obese than ob/ob controls and had markedly increased energy expenditure with reduced triglyceride storage in liver (Cohen *et al.* 2002). Thus, SCD-1 is an important component of the novel metabolic response to leptin signaling. Such studies are currently lacking in birds (non mammalian species) which manifest some peculiarities: (1) broiler chickens were selected for rapid growth and high food intake and are prone to obesity (Griffin & Goddard 1994); (2) leptin is expressed not only in adipose tissue, but also in liver in chickens (Taouis *et al.* 1998, Ashwell *et al.* 1999), while it is mainly expressed in adipose tissue in mammals (Zhang *et al.* 1994); (3) the mechanism of leptin action on food intake regulation in chickens is quite different from that described in mammals (Dridi *et al.* 2005a); and (4) as in human, lipogenesis occurs essentially in the liver of chickens (Leveille *et al.* 1968), however, in rodents, lipogenesis occurs in both adipose tissue and liver (Blair *et al.* 1991). Therefore, chicken is an interesting model for understanding appetite, satiety, and lipid metabolism at molecular levels.

The present study aimed, first, to determine the expression of SCD in different tissues of female and male broiler chickens; secondly, to investigate the effects of leptin and cerulenin on food intake and SCD gene expression in chicken liver, hypothalamus, and muscle; and finally, to assess whether food deprivation and genetic selection for abdominal fat pad size affect SCD gene expression in these metabolically important tissues.

Materials and Methods

Animals

Experiments were conducted in accordance with the directives of the European community (86/609/EEC) on the care and use of laboratory animals and the experimental protocols were approved by the K U Leuven Ethical Committee for Animal Experiments.

Experiment 1: tissue distribution of SCD gene expression in male and female broiler chickens

Male and female broiler (Cobb 500 strain) chickens (Avibel, Halle-Zoersel, Belgium) of 6 weeks of age (1765 and 2665 g for female and male respectively) were kept in floor pens under a 14 h light:10 h darkness cycle. Chickens were supplied with food (Table 1) and water available *ad libitum*. Three chickens from each gender were killed by cervical dislocation and tissues (adipose tissue, lung, liver, kidney, hypothalamus, heart, leg and breast muscle, pancreas, gizzard, proventriculus, intestine, testis, and ovary) were removed, immediately snap frozen in liquid nitrogen, and stored at -80 °C until use.

Table 1 Diet composition and calculated analysis

	Experiment 1, 2 and 3	Experiment 4
Ingredients (g/kg)		
Wheat	350.2	384.5
Yellow maize	150.0	100.0
French peas	150.0	120.0
Soybean meal, 500 g crude protein/kg	125.0	34.0
Full fat soy	100.0	100.0
Sunflower meal, 280 g crude protein/kg	—	5.0
Oilcake meal	20.0	55.0
Rapeseed meal	—	26.0
Animal meal	65.0	105.0
Fish meal	10.0	10.0
Animal fat	12.0	18.0
Fatty acids	—	10.0
Limestone	2.4	—
Methionine	2.3	1.3
Lysine	2.1	0.7
Choline	0.5	0.5
Common salt	1.5	—
Vitamin and mineral premix ^a	4.0 ^b	4.0 ^c
Wheat enzyme preparation	4.0	4.0
Sodium bicarbonate	—	1.0
Sepiolite	—	20.0
Calculated composition (g/kg)		
Crude protein	222.2	214.2
Crude fiber	29.7	31.5
Crude fat	58.2	81.3
Starch and sugars	428.4	408.7
Moisture	117.9	110.0
Lysine	11.0	10.4
Methionine and cystine	8.8	7.6
Tryptophan	2.2	2.1
Threonine	7.1	6.7
Choline	1.5	1.5
Essential fatty acids	19.9	24.3
Potassium	—	7.1
Calcium	9.0	7.5
P (total)	7.0	6.0
P (available)	4.9	4.3
Chloride	2.6	2.3
Sodium	1.5	1.9
Metabolizable energy (MJ/kg)	12.1	12.6
L-carnitine	17.8	22.9

^{a,b}Vitamin and mineral premix provided per kilogram diet: retinol acetate, 3800 µg; cholecalciferol, 60 µg; α-tocopherol, 30 mg; menadione, 1.5 mg; thiamin, 1.5 mg; riboflavin, 4 mg; pyridoxine, 2 mg; cobalamin, 20 µg; niacin, 30 mg; biotin, 70 µg; folic acid, 1 mg; pantothenic acid, 10 mg; Mn, 80 mg (MnO); Fe, 90 mg (FeSO₄·H₂O); Cu, 22 mg (CuSO₄·5H₂O); Co, 0.2 mg (CoSO₄); I, 0.8 mg (KI); Se, 0.2 mg (Na₂SeO₃); Zn, 50 mg (ZnO).
^{a,c}Vitamin and mineral premix provided per kilogram diet: the same as ^{a,b} except for retinol acetate, 3100 µg; menadione, 2 mg; thiamin, 2 mg; cobalamin, 120 µg; biotin, 50 µg; I, 0.54 mg (KI).

Experiment 2: leptin treatment

Male broiler chicks (Ross 308), which were 1 day old, were purchased from Avibel and reared in floor pens until 2 weeks of age, at which time the birds were transferred to individual cages and the diet in Table 1 was fed. After 3 days of adaptation, birds were weighed and cannulated in the brachial artery (Huybrechts *et al.* 1992) under local anesthesia (xylocaine). The chickens were allowed to recover and

adapt during 4 more days. Before the infusion experiment, the chickens were divided into two homogenous weight-matched groups ($n=5$, mean body weight was 1000 g) and food deprived for 2 h in order to increase their appetite. The mini pump (Syringe pump series, Model 22, Harvard apparatus, Holliston, MA, USA) infused recombinant chicken leptin prepared as previously described (Raver *et al.* 1998; 8 $\mu\text{g/kg/h}$) or saline at a constant rate of 3 ml/h during 6 h and food intake was recorded after the treatment (6 h). Birds were killed by cervical dislocation and tissues (hypothalamus, liver, and leg muscle) were removed, snap frozen in liquid nitrogen, and stored at -80°C until use.

Experiment 3: cerulenin administration

Broiler chickens (Ross 308), which were 1 day old, were purchased from a commercial hatchery (Avibel) and reared on floor pen until 1 week of age, at which time the birds were transferred to individual cages and provided with individual feeders and drinking nipples. Food (Table 1) and water were consumed *ad libitum* and the lighting schedule provided 14 h of light per day. After 1 week of adaptation, birds were divided into two homogenous weight- (267 g) and food intake-matched groups ($n=4$), and food deprived for 2 h in order to increase their appetite. Each bird received an i.v. injection (at 0, 4, and 24 h) of 15 mg/kg cerulenin (Sigma) or equal volume of vehicle (10% dimethyl sulfoxide in Roswell Park Memorial Institute Medium 1640 medium). Cumulative food intake was measured after 28 h and tissues (hypothalamus, liver, and leg muscle) were removed, frozen in liquid nitrogen, and stored at -80°C until use.

Experiment 4: genotype and nutritional status

In order to assess whether SCD gene expression is regulated by nutritional state and genotype, two broiler chicken lines were used. These two lines were established by long-term divergent selection for ratio of abdominal fatness to live weight, after which the fat line (FL) had about 1.5- to 2-fold abdominal fat weight of the lean line (LL) at 9 weeks of age (Leclercq *et al.* 1980). Chickens (male) of each line (mean body weights were 2400 and 2230 g for FL and LL respectively) were kept in conventional floor pens, fed *ad libitum* with a balanced diet (Table 1), and exposed to a daily 14 h light period. At 9 weeks of age, chickens were submitted to two different nutritional states: food deprivation for 16 or 24 h and feeding *ad libitum* ($n=3$). After blood sampling and cervical dislocation, tissues (hypothalamus, liver, and leg muscle) were quickly removed, frozen in liquid nitrogen, and stored at -80°C until use.

Reverse transcription and PCR (RT-PCR)

Total RNA was extracted from 100 mg tissue using the Trizol reagent (Invitrogen) according to the manufacturer's protocol. RNA integrity was assessed via 1% agarose gel electrophoresis and RNA concentrations and purity were determined for

each sample spectrophotometrically using UV absorbance (260/280). Total RNA (1 μg) was reverse transcribed and subjected to PCR in the presence of two pairs of primers. The first one was specific to chicken SCD (GenBank Accession number X60465): sense 5'-TCCCTTCTGCAAAGATC-CAG-3' and antisense 5'-AGCACAGCAACACCACT-GAG-3', flanking a 402 bp region. The second pair of primers was specific for chicken ribosomal 18S RNA (internal control; for sequences, see Dridi *et al.* 2005a). PCR was performed in 50 μl solution containing 2 μl RT product, 1 U Taq DNA polymerase (Roche Diagnostic), 0.1 mmol/l dNTP mixture, and 10 pmol of each forward and reverse primer. Twenty-five cycles (35 for characterization and probe preparation) were performed, each cycle consisting of denaturation (94°C , 30 s), annealing (58°C , 30 s), and elongation (72°C , 1 min) except for the first cycle in which denaturation was for 2 min and the last cycle in which the extension time was for 10 min. The number of cycles used for each gene was in the linear amplification range.

Probe labeling and Southern blot analysis

The amplified fragments were separated on a low melting point agarose gel (1%) and the appropriate bands were cut out, purified using Qiaquick gel extraction kit protocol (Qiagen) and stored at -20°C . The cDNA fragments were cloned in the pPCR Script Amp SK (+) cloning vector using the pPCR Script Amp cloning kit (Stratagene) and automatically sequenced using an Applied Biosystems automated sequencer. The cloned fragments (25–30 ng) were labeled by random priming with (α - ^{32}P) dCTP (Feinberg & Vogelstein 1983). The amplified PCR products were transferred to nylon membrane using a vacuum blotting apparatus (Amersham Biosciences) and cross-linked by u.v. irradiation and baked at 80°C for 20–30 min. Membranes were hybridized with heat denatured ^{32}P -labeled DNA probes, prepared as described earlier, at 42°C overnight. During the following day, the membranes were rinsed twice with $1\times$ SSC, 0.1% SDS at 55°C . Each wash was for 20 min and then membranes were subjected to storage phosphor autoradiography cassette. Hybridization signals were quantified using phosphorimager (Bio-Imaging Analyzer BAS 1000 Mac BAS, Fujix (Fuji, Tokyo, Japan), TINA software, version 2.09, Belgium).

Plasma leptin measurement

Circulating leptin concentrations were determined by RIA (multi-species leptin RIA kit, Linco Research Co. Mo, USA). The RIA has been validated for chicken leptin (Dridi *et al.* 2000a). Samples were assayed in a single assay and the intra-assay coefficient of variation was 6.3%.

Statistical analysis

The data were analyzed using the Student's unpaired *t*-test except the data from experiments 1 and 4, which were

analyzed by two-factor ANOVA with tissue and gender (experiment 1), and genotype and nutritional state (experiment 4) as classification variables. If ANOVA revealed significant effects, the means were compared by Student–Newman–Keuls multiple range test using the general linear model procedure of Statistical Analysis System (SAS) software (SAS Institute 2000, Version 8.1). Differences were considered significant at $P < 0.05$.

Results

Tissue distribution of SCD gene expression in male and female chickens

SCD gene was expressed in all tissues examined in female and male broiler chickens. Proventriculus was found to contain the highest amount of SCD mRNA, followed by ovary, kidney, hypothalamus, liver, and adipose tissue in female chickens. However, in males, the highest levels were observed in hypothalamus, leg muscle, pancreas, liver, adipose tissue, and testis. Interestingly, when tissues from the two genders were plotted together, females exhibited significantly ($P < 0.05$) higher levels of SCD mRNA in kidney (68%), breast muscle (111%), proventriculus (212%), and intestine (320%) compared with the male (Fig. 1). In contrast, hypothalamic SCD gene expression was significantly higher in male than in female chickens (39%; $P < 0.05$).

Effect of leptin on chicken SCD gene expression

Recombinant chicken leptin increased plasma leptin levels (23-fold; $P < 0.0001$) and reduced cumulative food intake (51%; $P < 0.05$) as compared with the control (Table 2). Despite the inhibition of food intake, leptin significantly

induced the expression of SCD gene in chicken liver as compared with the control (29%; $P < 0.05$; Fig. 2). However, the SCD mRNA levels in the hypothalamus and muscle were not affected by this treatment (1.13 ± 0.14 vs 1.00 ± 0.05 for hypothalamus, and 1.10 ± 0.10 vs 0.95 ± 0.10 for muscle of the control- and leptin-treated group respectively; mean $\pm$ S.E.M., $n = 5$).

Effect of cerulenin on chicken SCD gene expression

Cerulenin treatment reduced the cumulative food intake by 22% ($P < 0.01$) and plasma leptin levels by 22.5% ($P = 0.3$) relative to the control (Table 2). Cerulenin administration significantly reduced the expression of SCD gene in muscle by 37% ($P < 0.05$; Fig. 3), but not in liver or hypothalamus (0.82 ± 0.13 vs 0.95 ± 0.08 for hypothalamus, and 1.18 ± 0.19 vs 1.21 ± 0.05 for liver of control- and cerulenin-treated group respectively; mean $\pm$ S.E.M., $n = 4$).

Effect of genotype and nutritional state on chicken SCD gene expression

Food deprivation for either 16 or 24 h significantly down-regulated the hepatic SCD gene expression in FL by 52 and 52.5% respectively and in LL chickens by 43 and 34.7% respectively compared with that of their *ad libitum* fed counterparts ($P < 0.05$; Fig. 4A and B). However, the hypothalamic SCD mRNA levels were significantly decreased by 17% for FL and 13.3% for LL chickens only after 24 h of food deprivation compared with *ad libitum* feeding state ($P < 0.05$; Fig. 5). In muscle, food deprivation for either 16 or 24 h did not affect the expression of SCD. Furthermore, independent of the nutritional state, SCD gene expression did not differ between the two lines in all tissues examined.

Figure 1 Relative quantity of SCD mRNA in different tissues of 6-week-old female and male broiler chickens. Values are means $\pm$ S.E.M., $n = 3$. *Different from the female, $P < 0.05$.

Table 2 Effects of leptin and cerulenin treatments on cumulative food intake and plasma leptin levels in broiler chickens. Values are means $\pm$ S.E.M.

	Control group	Treated group
Leptin treatment		
Food intake (g/6 h)	67.1 $\pm$ 9.4	32.8 $\pm$ 3.9*
Plasma leptin levels (ng/ml)	2.68 $\pm$ 0.32	61.9 $\pm$ 4.7*
Cerulenin treatment		
Food intake (g/28 h)	85.9 $\pm$ 2.4	67.0 $\pm$ 1.9*
Plasma leptin levels (ng/ml)	1.29 $\pm$ 0.26	1.00 $\pm$ 0.05

*Different from the control, $P < 0.05$.

Discussion

SCD is expressed in a wide range of tissues in broiler chickens. The expression of SCD gene is tissue- and gender-dependent, corroborating previous studies in mammals (Lee *et al.* 1996, Chung *et al.* 2000, Zheng *et al.* 2001). The underlying mechanism(s) for these differences is unknown. It could be due to differences in fat deposition within tissues and/or levels of hormones, particularly sexual hormones. Estrogen administration induces SCD activity and causes a remarkable increase in plasma lipid and very low density lipoprotein production in avian species (Lippiello *et al.* 1979, Dashti *et al.* 1983). Several peroxisome proliferators induce SCD gene expression differently in male than in female and this difference has been shown to be related to higher levels of testosterone in male (Kawashima *et al.* 1989). It is possible that other hormones known to be involved in lipid metabolism, such as leptin (Cohen *et al.* 2002), ghrelin (Theander-Carrillo *et al.* 2006), growth hormone, and

Figure 2 Effect of recombinant chicken leptin infusion on hepatic SCD gene expression in 3-week-old broiler chickens. Values are means $\pm$ S.E.M., $n = 5$. *Different from the control, $P < 0.05$.

Figure 3 Effect of cerulenin administration on SCD gene expression in muscle of 2-week-old broiler chickens. Values are means $\pm$ S.E.M., $n = 4$. *Different from the control, $P < 0.05$.

thyroid hormones (Waters *et al.* 1997, Ameen *et al.* 2004), might explain the tissue-specific and sex-dependent expression of avian SCD (Kuhn *et al.* 1996, Richards *et al.* 2000, Liu *et al.* 2002).

Recent reports have clearly shown that SCD-1 is involved, at least partly, in the effect of leptin on energy expenditure and body weight (Cohen *et al.* 2002, Cohen & Friedman 2004). Leptin was found to reduce adiposity and liver triglyceride content in part by reducing SCD-1 mRNA and enzymatic activity in liver (Cohen *et al.* 2002). Such exciting data, which are currently lacking in non-mammalian species, prompted us to investigate the regulatory effects of leptin on SCD gene expression in three metabolically important tissues, namely the liver (the main site of lipogenesis (Leveille *et al.* 1968)), the hypothalamus (the site of food intake and energy homeostasis control (Robinson *et al.* 1975, Denbow 1985)), and the muscle (the main site of thermogenesis (Duchamp & Barré 1993)).

The biological activity of the recombinant chicken leptin was demonstrated previously by its ability to stimulate the proliferation of BAF3 cells *in vitro*, transfected with the functional long form of the human leptin receptor (ob-Rb), and *in vivo* by its inhibitory effect on food intake after a single injection (Raver *et al.* 1998, Dridi *et al.* 2000b). In the present study, recombinant chicken leptin administered continuously increased plasma leptin levels and inhibited food intake. Surprisingly, despite the decrease in food intake, leptin significantly increases SCD gene expression in chicken liver, but not in hypothalamus or muscle. These results indicate that leptin regulates SCD gene expression in a tissue-specific manner.

Figure 4 Effect of (A) 16 or (B) 24 h food deprivation on hepatic *SCD* gene expression in genetically fat and lean line chickens. Values are means $\pm$ S.E.M., $n=3$. Superscript letters indicate a significant difference at $P<0.05$. Genotype effect (G), $P>0.05$; nutritional state effect (NS), $P<0.05$; interaction (G $\times$ NS), $P>0.05$. F, fed; FD, food deprived.

The absence of leptin's effect on hypothalamic and muscle *SCD* gene expression should be interpreted cautiously because other specific *SCD* isoforms, that are not yet known, may exist and may be affected by leptin as previously reported for mammalian heart *SCD-4* (Miyazaki *et al.* 2003). In addition, in our study, we measured only RNA levels and leptin may affect the enzymatic activity differently as previously reported for mammals (Miyazaki *et al.* 2003).

Leptin seems to have an opposite effect on chicken hepatic *SCD* gene expression compared with that described in mammals (Cohen *et al.* 2002). The mechanisms behind this difference are unclear and may be related to many factors such as dose and time of leptin treatment and species-specific effects. It has been shown that leptin exerts a dose-dependent biphasic effect on mammalian steroidogenesis (Ruiz-Cortes *et al.* 2003) and corticosterone secretion (Malendowicz *et al.* 2004). In mammals, leptin is mainly expressed in adipose tissue (Zhang *et al.* 1994) and its effects on hepatic *SCD-1* are probably mediated by central action (Cohen *et al.* 2002). In birds, leptin is expressed not only in adipose tissue, but also in liver (Taouis *et al.* 1998, Ashwell *et al.* 1999), which is the major source for leptin (Richards *et al.* 1999). Although leptin inhibits the expression of its receptor in liver and hypothalamus (Dridi *et al.* 2005a,b), the specific mechanism by which leptin induces *SCD* gene expression in chicken liver is presently unknown and further studies are warranted.

Although leptin has long been known to play roles in the regulation of food intake and energy homeostasis, the potential role of fatty acid metabolism in this process has been considered only recently. Inhibition of fatty acid synthesis by cerulenin reduces food intake and induces profound reversible weight loss (Loftus *et al.* 2000). Centrally, this compound was hypothesized to alter, like leptin, the expression profiles of feeding-related neuropeptides, often

inhibiting the orexigenic and inducing the anorexigenic neuropeptide gene expression (Shimokawa *et al.* 2002). We have recently shown that cerulenin inhibits food intake by altering, like leptin, the expression of melanocortin receptors, but without modulating the other known neuropeptides that are involved in food intake regulation (Dridi *et al.* 2006). Therefore, we sought to assess in this study whether cerulenin

Figure 5 Effect of food deprivation (24 h) on hypothalamic *SCD* gene expression in genetically fat and lean line chickens. Values are means $\pm$ S.E.M., $n=3$. Superscript letters indicate a significant difference at $P<0.05$. Genotype effect (G), $P>0.05$; nutritional state effect (NS), $P<0.01$; interaction (G $\times$ NS), $P>0.05$. F, fed; FD, food deprived.

affects, like leptin, *SCD* gene expression. Our data showed that cerulenin administration reduces food intake and decreases *SCD* mRNA levels in chicken muscle. However, the hepatic and hypothalamic *SCD* gene expression was not affected by cerulenin treatment. This result supports again the tissue-specific regulation of *SCD* and suggests that leptin and cerulenin may regulate *SCD* gene expression via different pathways.

Many developmental, dietary, hormonal, and environmental factors regulate *SCD*-1 gene expression (Miyazaki & Ntambi 2003). We ascertain in the present study whether *SCD* mRNA levels differ between tissues in response to starvation and abdominal fat pad size of FL and LL chickens. The expression of *SCD* is reduced by either 16 or 24 h food deprivation in chicken liver, but not in muscle. Furthermore, food deprivation for 24 h, but not for 16 h, downregulates *SCD* mRNA levels in chicken hypothalamus. These data indicate that the sensitivity of *SCD* gene to food deprivation varied among tissues, with the highest sensitivity in the liver. The mechanism behind the time-lag between tissues is still unknown. The repression of *SCD* gene expression in the hypothalamus by food deprivation suggests that hypothalamic *SCD* responds to feeding status and may be involved in the regulation of food intake in chickens as previously described in mammals (Ntambi *et al.* 2002, Dobrzyn & Ntambi 2004, Guoqiang *et al.* 2005). Regardless of the nutritional state, *SCD* mRNA levels did not differ between FL and LL chickens. This result is concordant with the previous finding concerning hepatic *SCD* mRNA (Daval *et al.* 2000). However, previous studies have shown that hepatic *SCD* activity was significantly higher in FL than in LL chickens (Legrand *et al.* 1987, Legrand & Hermier 1992). Furthermore, the proportion of palmitoleic acid which results from hepatic $\Delta 9$ desaturation was higher in FL compared with LL chickens (Legrand & Hermier 1992) and a positive correlation has been found between *SCD* mRNA levels and fatness *in vivo* (Douaire *et al.* 1992). We have recently shown that circulating leptin levels and hepatic leptin gene expression were significantly higher in FL compared with LL chickens (Dridi *et al.* 2005b). These observations in combination with the present data suggest that the interaction of leptin with *SCD* may be crucial in the regulation of avian hepatic lipogenesis.

In conclusion, the present study is the first to report the regulation of *SCD* gene expression in different metabolically important tissues by leptin, cerulenin, nutritional state, and gender. These factors regulate the expression of *SCD* gene in a tissue-selective manner. In contrast to mammals, leptin induces *SCD* gene expression in chicken liver, suggesting subtle species-dependent differences in the role of leptin at least in this tissue. Cerulenin inhibits *SCD* gene expression in muscle; however, food deprivation decreases *SCD* gene expression in both liver and hypothalamus of chickens and this effect was observed with a time-lag between tissues. The tissue-specific and sex-dependent expression of *SCD* suggests the presence of complex hormone-specific control mechanisms.

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