Special Issue: Advanced Themes in Endocrinology
Review

Avian Leptin: Bird's-Eye View of the Evolution of Vertebrate Energy-Balance Control

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Discovery of the satiety hormone leptin in 1994 and its characterization in mammals provided a key tool to deciphering the complex mechanism governing adipose tissue regulation of appetite and energy expenditure. Surprisingly, despite the perfectly logical notion of an energy-storing tissue announcing the amount of fat stores using leptin signaling, alternate mechanisms were chosen in bird evolution. This conclusion emerged based on the recent discovery and characterization of genuine avian leptin – after it had been assumed missing by some, and erroneously identified by others. Critical evaluation of the past and present indications of the role of leptin in Aves provides a new perspective on the evolution of energy-balance control in vertebrates; proposing a regulation strategy alternative to the adipostat mechanism.

Characterization of Leptin in Birds Sheds New Light on the Evolution of the Leptin Circuit and Adipose Tissue as an Endocrine Organ in Vertebrates

The regulation of food intake is essential for survival in all organisms but has been studied in depth primarily in mammals. The discovery and characterization of the satiety hormone **leptin** [1] (see Glossary) provided a huge breakthrough in understanding the molecular control mechanism of **energy balance**. This study also revolutionized the way in which we understand **the role of adipose tissue**, by enforcing its central endocrine role in all aspects of energy-balance control. A Southern blot in the first publication of leptin [1] suggested its conservation in all vertebrates. It was logical to assume that the roles of leptin and the adipose tissue are conserved in all vertebrates. However, the discovery and characterization of leptin in Aves provided some unexpected results.

Birds are an important model in evolutionary biology, representing over 30% of known tetrapod diversity with more than 10 700 living species (https://www.worldbirdnames.org/updates/). Being hot-blooded as part of their unique adaptation to flight, **bird evolution** bridges the gap between mammals and cold-blooded vertebrates. Birds are also important for agricultural research by providing some of the prominent livestock species that affect global economy and human health. Therefore, following the discovery of the key role of leptin in the control of energy balance in mammals, it became of high interest to characterize leptin activity in avian species as well. Further study revealed many differences in gene expression of leptin and its receptor, *LEPR*, compared to mammals, suggesting a different role. It appears that the **adipostat activity** of leptin, in controlling appetite and energy expenditure, may be disadvantageous for species with a critical need to accumulate a large amount of fat before migration/winter or for other seasonal needs.

Discovery of Leptin Genes in Avian Species

Leptins were discovered and characterized in birds [2–7] about two decades after the mammalian leptins [1], and about 10 years after finding and characterizing leptins in fish (*Takifugu rubripes* [8]), frog (*Xenopus laevis* [9]) and tiger salamander (*Ambystoma tigrinum* [10]). In retrospect, it is clear that leptin identification in avian species was hampered by the extreme guanine–cytosine (GC) content of about 70% and low sequence conservation for both the nucleic and amino acid sequences ($\lesssim$30%) (Figure 1), as well as low expression level (<0.5 reads per kilobase per million mapped reads; **RPKM**), evident in next-generation RNA sequencing (RNA-Seq) datasets [6,11]), and an unexpected tissue-expression profile ([2–7]; Figure 2, Key Figure). The recent explosion of submitted raw data from high-depth genomic sequencing [12] and RNA-Seq projects in birds enabled the identification of leptin, despite the indicated technical difficulties. These datasets were used independently in several laboratories for manual tailoring of putative leptin sequences from pieces of unannotated sequences in the GenBank Sequence Read Archive (SRA). The identified sequences were verified by

Highlights

Unlike their mammalian orthologs, bird leptins show no expression in adipose tissue.

Besides generally undetectable leptin in avian blood circulation, mRNA expression overlap of leptin and *LEPR*, and dominant expression of the long form of *LEPR* mRNA in all *LEPR*-expressing tissues (vs the short forms in mammals implicated in interactions with blood-circulating leptin), indicate that in birds, leptin does not operate as a hormone.

Although the adipostat role of leptin seems to have emerged in the common ancestor of amphibians and mammals, different roles have likely been adopted for leptin in birds to suit their specific lifestyles.

Extreme GC content of genes encoding avian adipokines has hampered evolutionary studies of energy-balance control in vertebrates; now that the controversy of the erroneously identified leptins is over, related reports can be critically evaluated.

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several complementary criteria [2–6], including PCR sequencing, similarity of gene structure and secondary and tertiary structure of the predicted proteins, and phylogenetic analyses, which placed the bird's leptin sequences (both mRNA and predicted protein) at the expected position between reptiles and mammals (Figure 3). The final proof of these identifications was a demonstration of local synteny with the closest gene neighbors of leptin. For zebra finch (*Taeniopygia guttata*) and budgerigar (*Melopsittacus undulates*) leptins, *Mir 129-1* was identified at the 5′ end of leptin genes [2]. For the saker falcon (*Falco cherrug*), *RBM28* was located at the 3′ end of the genomic sequence [4]. For peregrine falcon (*Falco peregrinus*) and saker falcon, *SND1*, *LRRC4*, *Mir 129*, *RBM28*, *IMPDH1*, *ATP6V1P*, and *FLNC* were mapped near leptin [5]. All of these identifications were made by sequencing the complete genomic scaffolds containing the leptin genes, which can lead to the identification of neighboring genes in birds due to the condensed structure of their genome, with a relatively short intergenic sequence compared to other vertebrates [13]. For example, *Mir 129-1* and *RBM28*, which in humans are placed at a distance of about 40 kb from leptin, are located just a few kilobases away from leptin in zebra finch and budgerigar [2], and about 1 kb away in saker falcons (*RBM28* [4]). Additionally, chicken leptin and syntenic genes were mapped to the chicken genome using a radiation hybrid panel, revealing their location at the distal tip of chromosome 1p [7].

Interestingly, the high GC content (about 70%) was observed not only in the avian leptin sequences, but also in the sequences of their syntenic regions [5,7]. The discovery of leptin in birds (Box 1) promoted the recent finding of other missing genes in avian species [14–17], altogether suggesting that other genes considered lost in chicken [18,19] may be hidden in GC-rich regions of the chicken genome, which we referred to as the dark side of the genome [6].

Leptin Receptor

The chicken *LEPR* gene was discovered in the year 2000 as the first *LEPR* in nonmammalian vertebrates [20,21]. The identification was confirmed by mapping to a syntenic region on chicken chromosome 8 [22] and by characterization of similar *LEPR*s in turkey, duck, goose, and quail with about 90% sequence similarity [23–25]. The sequences of bird *LEPR*s have a normal GC content (~45%, Figure 1C) and a higher similarity than leptin to the corresponding mammalian orthologs (~60%). The structure of the avian *LEPR* genes is highly similar to that of mammalian *LEPR*s, consisting of 20 exons with conserved boundaries [20,23]. The predicted protein sequence of the avian *LEPR*s indicates conservation of functional domains and consensus motifs of the mammalian *LEPR*s, belonging to the type 1 helical cytokine receptor superfamily [26]. These include the immunoglobulin-like C2-type domain, three fibronectin type 3 domains, two WSXWS (tryptophan–serine–X–tryptophan–serine) motifs, a single transmembrane segment, three consensus sequences named Boxes 1–3, and three tyrosine residues at conserved positions of the intracellular domain. The leptin-binding domain, characterized in mammals [27], is the most conserved region in avian LEPRs, with ~80% identical amino acids [28,29]. This domain of the chicken LEPR was also studied *in vitro*, demonstrating its leptin-binding capacity [30]. In mammals, the conserved motifs in the LEPR intracellular domain were implicated in the activation of the JAK/STAT and the PI3/AMPK pathways [26,31,32], as well as in a feedback mechanism that reduces leptin sensitivity through suppressor of cytokine signaling (SOCS)3 [33]. The signal-transduction capacity of the chicken *LEPR* was demonstrated using cell culture systems exogenously expressing the chicken *LEPR* [28,29,34]. Leptin administration to the culture medium of these cells induced STAT3 phosphorylation, and activation of a reporter gene placed under a STAT3-responsive promoter. Activation of the reporter gene in cell culture systems by a native or heterologous leptins was similar in cells harboring exogenous human, chicken [28], or frog *LEPR*s [9]. These findings are consistent with the suggested conservation of leptin structure and the leptin-binding domain in LEPRs among vertebrates [2,4,30,35,36]. However, no study has yet compared the effect of endogenous and heterologous leptins *in vivo*.

Leptin Diversification in Amino Acid Sequence and Function

The predicted protein sequence of avian leptins (Figure 1) shows low sequence similarity not only to their mammalian and reptilian orthologs (~30% and ~35%, respectively), but also among birds. For

Glossary

Adipokine: a regulatory factor secreted by the adipose tissue having autocrine, paracrine, and/or endocrine activity.
Adipostat activity: activity affecting both energy intake and energy expenditure, aimed at achieving a constant amount of energy stores in the body. The classical factor with adipostat activity is the mammalian leptin.
Bird evolution: birds evolved from tetrapod dinosaurs in the middle of the Mesozoic era, about 150 mya. The common ancestor with mammals is estimated to have appeared ~310 mya and with reptiles, ~250 mya [101–103].
Energy balance/homeostasis: the relationship between the energy obtained by food intake and the energy spent by the various activities of the body, including thermoregulation, physical activity, reproduction, immune response, and virtually all other biological processes.
Leptin: the mammalian satiety hormone produced almost exclusively by the adipose tissue, which signals the amount of fat stores and affects appetite, insulin sensitivity, inflammation, reproduction, immune response, and other aspects of energy expenditure.
Role of adipose tissue: the most fundamental role of adipose tissue is storing excess energy as fat in times of positive energy balance and releasing it in times of negative energy balance. However, characterization of leptin and other adipokines has revealed that the adipose tissue additionally possesses an essential role in energy homeostasis: The 'energy keeper' also announces the amount of fat stores to the brain and other tissues, thereby affecting the control of energy intake and expenditure.
RPKM: a commonly used metric for quantitating next generation sequencing data. This unit of transcript expression normalizes RNA-Seq data with respect to the length of the RNA transcripts and the sequencing depth of the samples. The average RPKM of chicken transcripts in RNA-Seq experiment is about 28 [104], and the RPKM value of one of the

example, chicken and duck leptins share 45% identical amino acids, whereas human and mouse, with similar evolutionary distance (estimated as 80 and 90 million years, respectively; http://www.timetree.org), share 83% amino acid identity [6]. In addition to sequence diversification, there are also deletions and insertions, such as the additional noncoding exon at the 5′ end of dove and zebra finch leptins [2,4], an extended amino acid at the N-terminal position of the chicken leptin [11], and quail, and other insertions and deletions of predicted amino acids (Figure 1A). The rapid evolution of the non-mammalian leptin amino acid sequences and the slower evolution of the corresponding *LEPR*s [36] suggest that leptin amino acid diversification does not relate to LEPR binding or activation but to interactions with other proteins. These may include regulatory factors that bind to leptin and affect its activity and tissue specificity, as has been suggested for deep diving seals *(Halichoerus grypus* and *Phoca vitulina*), which have specific respiratory challenges associated with diving. Seal leptin is expressed in the lungs and seems to have a role in pulmonary surfactant production as an adaptation to deep water diving [37]. It is possible that the surfactant role of seal leptin is played additionally to adiposity signaling, since seal leptin is expressed in the blubber where fat is stored. However, this has not been yet fully resolved [38]. Another example of a concomitant divergence in leptin sequence and role in mammals is the leptin of plateau pika (*Ochotona curzoniae*). This rodent has adapted to low ambient temperatures and its leptin has a higher capacity for conferring adaptive thermogenesis compared to human leptin, in addition to satiety signaling [39].

Therefore, it is tempting to speculate that in birds, sequence divergence of the leptin gene compared to other vertebrate clades and among birds also relates to diverged expression profile and role, possibly requiring interaction with proteins additional to LEPR. Thus, it would be of great interest to compare RNA-Seq analyses of multiple tissues of different bird types, such as chicken and duck.

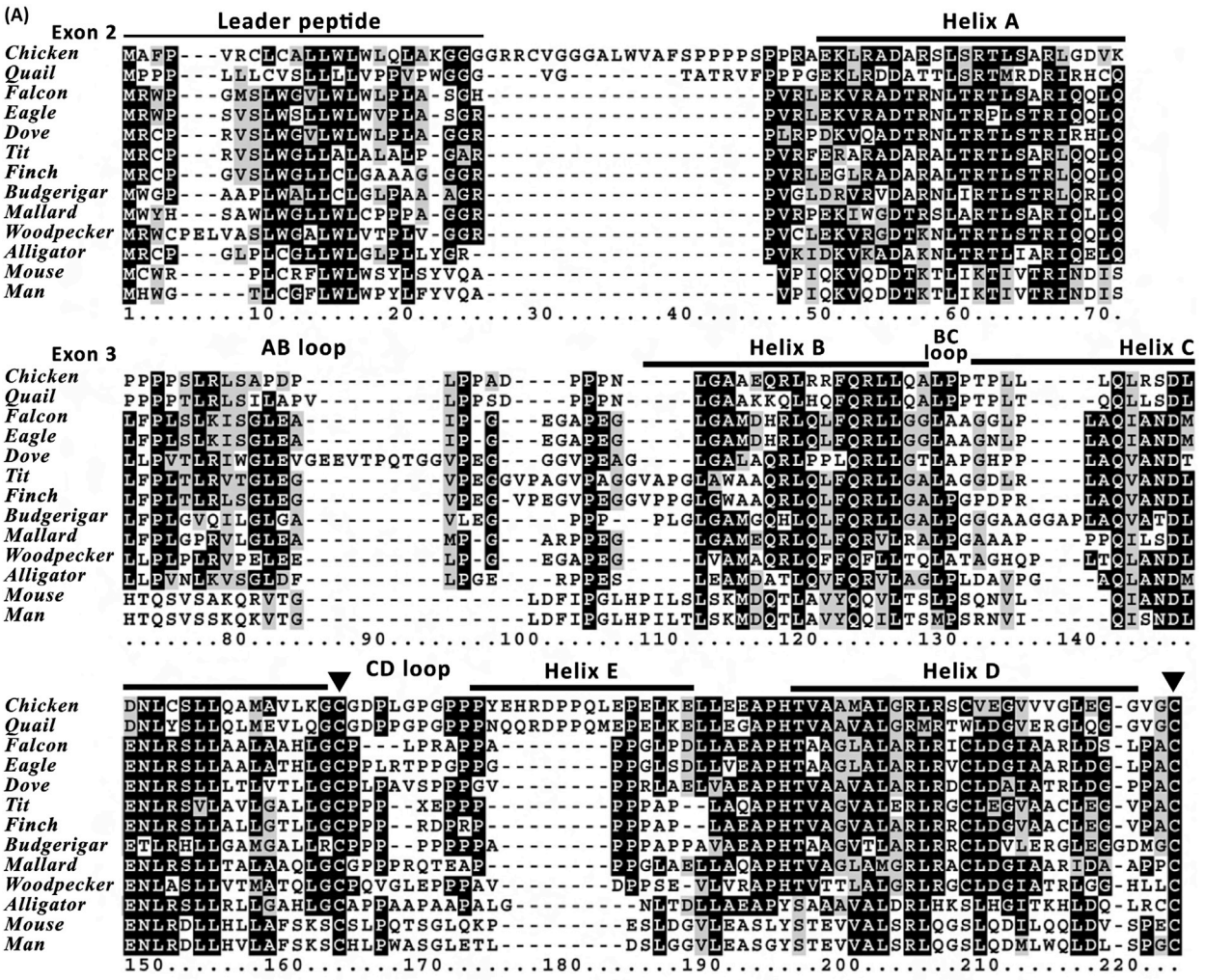
classical housekeeping genes GAPDH, is about 4000 [105]. Therefore, values below 1 RPKM are considered low.

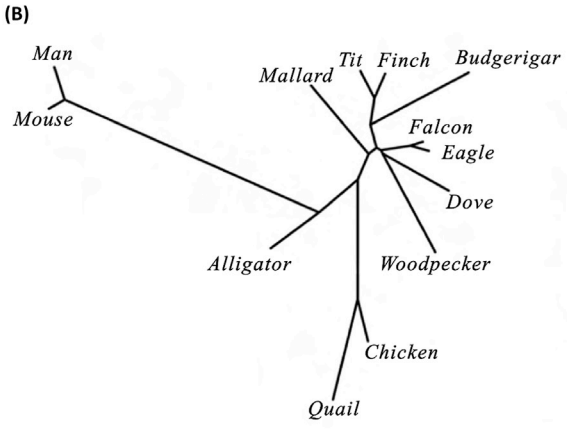
Aves Leptin Is Not an Adipokine

The most striking feature of the genuine avian leptins is that in sharp contrast to mammals, they are not expressed in the adipose tissue. The first indication came from quantitative PCR profiling of leptin mRNA expression in tissues of zebra finch, where it was detected in brain tissues and the pituitary, but was absent from peripheral tissues, including visceral fat [2]. This was confirmed by RNA-Seq analyses showing the absence of leptin mRNA (RPKM $\leq$0.01) in the visceral fat of the wild chicken red junglefowl [6,11], and two main commercial chicken lines (broilers and layers) that differ in appetite, body growth rate, and reproduction efficiency [40]. Moreover, there was no effect on leptin expression [40] in visceral fat in broiler chickens bidirectionally selected for either fast or slow body growth [41], or lean and fat phenotype [42]. In addition, leptin mRNA expression in the adipose tissue was not affected in chickens by 24-h feed deprivation [40]. Therefore, it is clear that adipose tissues in chicken do not signal the amount of fat stores *via* leptin. This seems to be true for other birds as well, based on the lack of leptin expression in the adipose tissue of zebra finches [2], and the low to undetectable leptin levels in the visceral fat of rock dove [4] and quail [6].

Is There Another Adipokine Replacing Leptin or Does Adipose Tissue Have a Different Role in Birds?

A search for adipokines that are positively correlated at the mRNA level to fat accumulation and food intake in RNA-Seq data of the various aforementioned chicken models failed to show a candidate that might replace the adipostat function of leptin [40]. Instead, this study revealed that three prominent adipokines (*TNF*, *IFG* and *IL6*) – which in mammals operate together with leptin in the control of appetite, insulin resistance, and inflammation – are expressed at low levels in chicken visceral fat. Similar to leptin, these three adipokines were also studied under a variety of physiological conditions relevant to obesity, growth, and reproduction efficiency; they showed no differential mRNA expression, and they were below the level of detection by mass spectrometry [40]. This suggests that the initial finding of the absence of leptin expression in adipose tissue may represent a more profound difference in adipose endocrine role in Aves.





(C)

Species	Leptin GC content	Average	LEPR GC content	Average
Chicken (*Gallus gallus*)	67%		41%	
Japanese quail (*Coturnix japonica*)	66%		42%	
Masked bobwhite quail (*Colinus virginianus*)	77%		44%	
Mallard (*Anas platyrhynchos*)	74%		49%	
Bald eagle (*Haliaeetus leucocephalus*)	73%		39%	
Peregrine falcon (*Falco peregrinus*)	74%	74%	39%	43%
Zebra finch (*Taeniopygia guttata*)	81%		47%	
Ground tit (*Pseudopodoces humilis*)	82%		45%	
Downy woodpecker (*Picoides pubescens*)	62%		45%	
Rock dove (*Columba livia*)	77%		42%	
Budgerigar (*Melopsittacus undulatus*)	80%		41%	
Green sea turtle (*Chelonia mydas*)	54%		39%	
Green anole lizard (*Anolis carolinensis*)	61%		37%	
Mouse (*Mus musculus*)	56%		44%	
Platypus (*Ornithorhynchus anatinus*)	46%	52%	49%	41%
Human (*Homo sapiens*)	58%		40%	
African clawed frog (*Xenopus laevis*)	43%		37%	
Zebrafish (*Danio rerio*)	45%		44%	

Trends in Endocrinology & Metabolism

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Key Figure

Comparison of Leptin and Long-Form LEPR Expression Profiles in Mammals (Woman's Image) versus Birds (Chicken's Image)

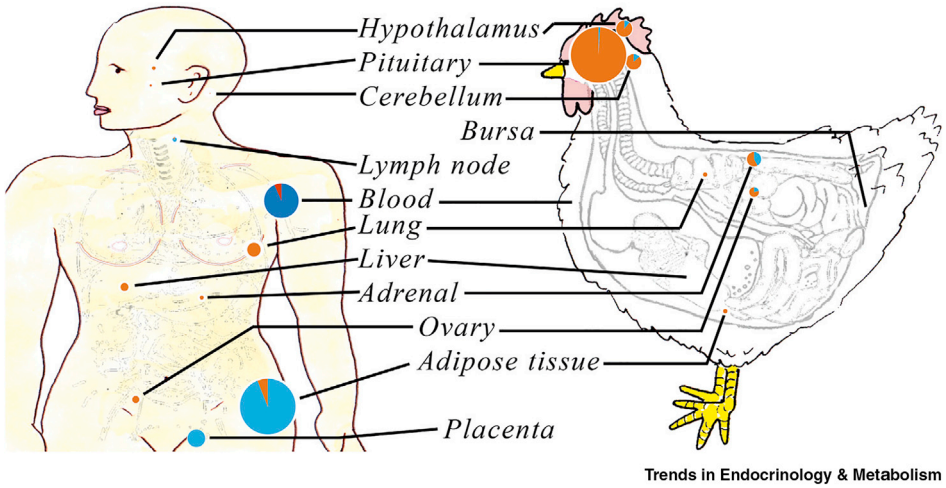

Figure 2. Pie charts denote expression based on RPKM data for mammalian leptin (light blue) and for long-form *LEPR* (*LEP-Rb*; orange) mRNAs, which were taken from representative RNA-Seq datasets of baboon (*Papio anubis*), monkey (*Rhesus macaque*; ovary) and woman (placenta) (GenBank accession nos. SRP107218, ERP108988 and SRX083287, respectively). In the blood, leptin (~28 ng/ml, dark blue) and soluble LEPR (~2 ng/ml; red) protein levels are from women with BMI~28 [128]. The data for Aves mRNA expression (color codes follow that of the mammals) were from RNA-Seq data of red junglefowl and Chinese chicken breeds (pituitary) [6]. Due to much lower expression levels, pies representing avian mRNA are drawn at a 12-fold larger scale than those of mammals. In mammals, leptin is primarily expressed in the adipose tissue and secreted into the blood. Mammalian *LEP-Rb* mRNA is detected in several peripheral tissues, which express primarily short forms of *LEPR* (not shown), whereas in the hypothalamus, the long form is dominant. In birds, leptin is expressed in brain tissue, adrenal glands and gonads, and is not generally detectable in the blood. In birds, the long form is the principal form of *LEPR* in all expressing tissues, and there is no indication of soluble forms in the blood. Unlike mammals, in birds, leptin and *LEPR* are correlatively co-expressed. However, *LEPR* is dominantly expressed in the pituitary. Differences in expression patterns between the two clades indicate evolutionary divergence in the role of leptin.

Obesity and Insulin Resistance Are Not Connected in Chickens

The mRNA profiling of leptin, *TNF*, *IFNG*, and *IL6*, suggested that in birds, adipose tissue does not control appetite, insulin resistance, or inflammation. This suggestion is strongly supported by surprising results obtained more than 30 years ago. Simon, Leclercq, and colleagues bred broiler chickens for high and low fat accumulation at similar body weights and found that the fat-line chickens had hypoglycemia, slight hyperinsulinemia, and seemed to be even more sensitive to insulin than the lean line were [43,44]. Moreover, while the difference in obesity was maximally manifested in the

Figure 1. A Bird's-Eye View of Leptin Sequences.
(A) Alignment of leptin amino-acid sequences. Dashes indicate gaps introduced by the alignment program (CLUSTALW). Identical and similar amino-acid residues in at least three or seven sequences are indicated with a black and gray background, respectively. White boxes indicate non-conservative amino-acid changes between proteins. Signal peptide and structural elements, helixes and loops, based on the crystal structure of human leptin [126], are denoted above the alignment. The two conserved cysteines forming a lasso knot [127] are indicated by black arrowheads. (B) Phylogenetic tree. (C) Comparison of *LEP* and *LEPR* GC content in birds versus other vertebrates. Sequences follow those published by Seroussi *et al.* ([6]; Table 3), except for those of *Alligator mississippiensis* and *Coturnix japonica* (GenBank accession nos. KYO26360 and MK689854, respectively).

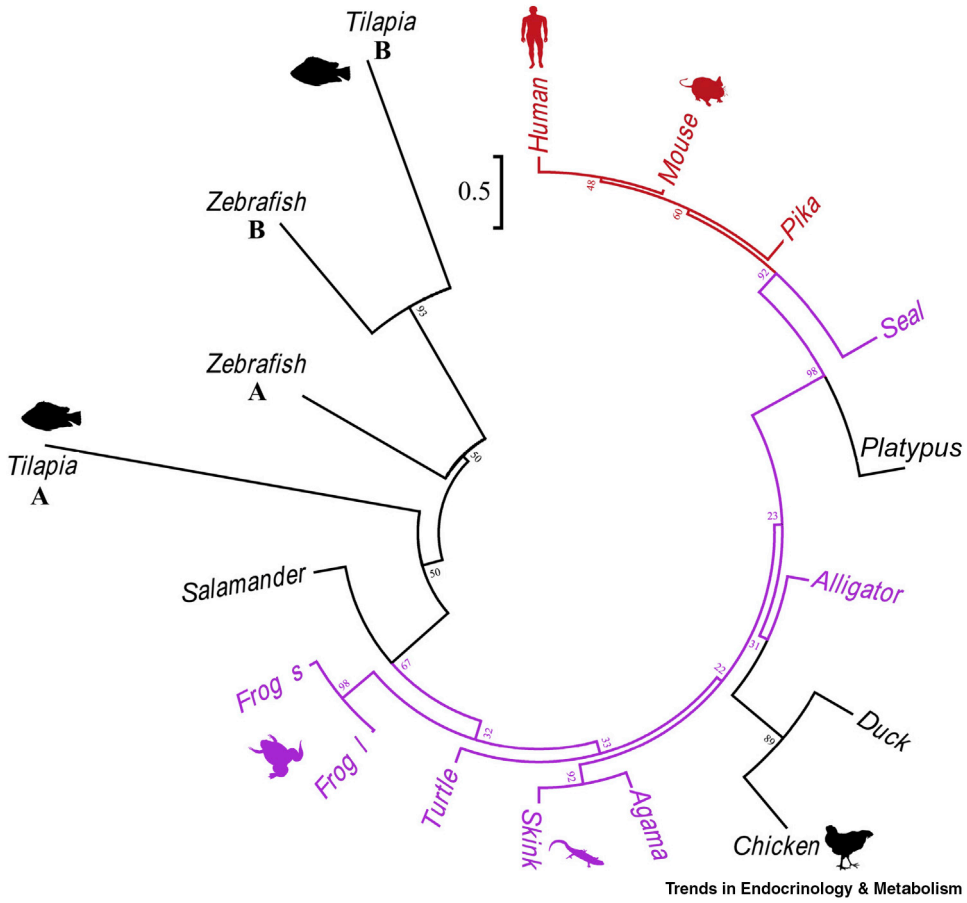

Figure 3. Phylogenetic Tree Based on the Predicted Amino Acid Sequence of Vertebrate Leptins.
The evolutionary history was inferred using the neighbor-joining method and is drawn to scale using the model with the lowest Bayesian-information criterion (JTT method, gamma 1.92). Gaps were eliminated and the final dataset had 107 positions. The corresponding GenBank accession numbers are given below, in a clockwise order, where applicable. The tree was inferred from 500 bootstrap trees and for each branch the percentage of replicate trees, which contained identical branch is shown. Evolutionary analyses were conducted in MEGA6 [129]. Pika (XP_004592562) and seal (XP_006747941) leptins are placed relatively far from human and mouse despite the similar evolutionary time of divergence of about 90 MYA among the four species (TIMTREE, http://www.timetree.org). Branch length is also larger between the bird's leptins comparing to that of human and mouse. This high sequence diversification has been suggested to relate to variation in leptin's expression pattern and role. Colors refer to the strength of the evidence for leptin adipostat activity. In the majority of mammals, leptin is a satiety signal working in a feedback loop (red). In seal, frog (XP_018108304; NP_001089183), turtle (XP_007052942), alligator (XP_014465773) and lizards (XP_020659433; XP_028602273), the adipostat role of leptin has been suggested but not fully established or is not known (pink). In teleost fish (XP_003440618; XP_009291735; NP_001025357; XP_005451664), birds (NP_001337009; APC23099), salamander (AAY68417) and monotrem marsupials (platypus, XP_001509133) there are strong evidence against adipostat role of leptin (black).

divergently bred lines at 9 weeks of age, glucose clearance remained faster in the fat line also at 17 weeks of age [44]. The authors termed this observation glucose–insulin imbalance, since the higher insulin level in the fat line was correlated with higher insulin sensitivity, in sharp contrast with the tight link between hyperinsulinemia and insulin resistance in obese mammals [45]. Following this up, broiler chickens were bidirectionally selected for high and low blood glucose levels [46]. The low-glucose breed accumulated more visceral fat, confirming that in chicken, higher sensitivity to insulin

Box 1. Correct Identification of Avian Leptin Is Evident

Despite the low primary sequence conservation of the newly identified leptins in avian species, their identification is unequivocal. Conservation of gene structure and of the secondary and tertiary structures of the predicted protein products, correct phylogenetic position and chromosomal mapping to syntenic regions in the corresponding genomes all support these identifications. This discovery finally put an end to the controversy over the erroneous sequence of chicken leptin (GenBank accession no. AF012727). This erroneous leptin cloned by PCR [106,107] had more than 95% similarity to mouse leptin at both the protein and nucleic acid levels, and was never included in chicken genomic assembly or located within a genomic contig. In phylogenic analyses, the erroneous leptin sequence was placed – counter to Darwin's evolutionary theory – at closest proximity to mouse [4,5]. The radioimmunoassay that was developed based on this erroneous sequence [57] and is still in use today [59] led to controversial results.

leads to increased fat accumulation with no major countereffect of obesity on insulin sensitivity. While the etiology of fattening in mammals relates to leptin and insulin resistance, in chickens it seems to be due to higher sensitivity to insulin. This early work predicted lack of adipostat activity in chickens, since energy expenditure as measured by body temperature was similar in the fat and lean lines [43], while in mammals leptin acts to modulate energy expenditure towards balancing energy stores [47].

Leptin and *LEPR* mRNA-Expression Profiles

Profiling of leptin mRNA by quantitative PCR and RNA-Seq in a variety of tissues in avian species, including zebra finch, rock dove, quail, and chicken, reveals its expression in the cerebellum, hypothalamus, pituitary, adrenal glands, and gonads (in descending order of expression levels) [2,4,6,40]. In the vast majority of the RNA-Seq studies (except for sporadic cases of liver studies; Box 2), leptin expression level was low (<0.5 RPKM [6]), even in the top-producing brain tissues. It is possible that this low leptin level in avian tissues, indicated by RNA-Seq, reflects the technical difficulty and the actual level of expression is thus higher. Therefore, studies at the protein level would be of high value for profiling leptin expression in birds.

The mRNA-expression profile of avian *LEPR* is tightly correlated to that of leptin [2,4,6], with generally higher *LEPR* mRNA levels (~3-fold) compared to leptin, suggesting a paracrine/autocrine mode of

Box 2. No Leptin Expression in Liver, with Sporadic Exceptions

The liver is the primary leptin-producing tissue in many nonmammalian nonavian vertebrates [81], compatible with being a prominent site of energy metabolism and storage, along with the adipose tissue. However, most of the experimental data show no leptin expression in the liver of birds. A search of RNA-Seq data in GenBank SRA with avian leptin sequences as bait revealed no leptin mRNA in the liver of red jungle fowl, rock dove, quail, or Faiyumi or broiler chickens exposed to various levels of heat stress, or in fatty or lean livers of Jingxing-Huang chickens. However, two experiments in duck liver found the expression of exceptionally high leptin mRNA [6] – 12 and 4 RPKM, respectively – which are the highest levels detected for leptin mRNA in any bird tissue in GenBank. Most quantitative PCR analyses of leptin mRNA in livers of zebra finch, quail, and chicken revealed little or no leptin [2,6,40]. A few exceptions were reports of leptin expression in the liver of rock doves captured in the city [4] and an experiment with mature female quails [25]. In another report in mature female quails, leptin was observed in only 1 out of 18 birds [6]. These observations indicate that in avian species, expression of leptin in the liver is sporadic and rare. Its expression may result from induction by a specific pathogen or reflect a specific biological cycling or metabolic situation. Reports on elevated leptin level in the blood circulation of hepatitis-C-virus-infected humans [108–110] demonstrate induction of leptin by pathogens. Taken together, the general observation is no leptin mRNA expression in bird liver, aside from the exceptionally high mRNA levels (>4 RPKM) in sporadic subjects. Because this mRNA expression exceeds >20-fold the normal expression in other leptin expressing tissues, it may reflect a fundamental feature of the role of leptin in birds.

Box 3. Short Forms of Avian *LEPR*

In mammals, six forms of *LEPR* mRNA (*LEPRa–f*) are generated by alternative splicing. The long form (*LEPRb*), which has the full capacity of signal transduction, is expressed predominantly in the hypothalamus [26,32,111]. The other leptin receptor isoforms have the same extracellular leptin-binding domain at the N terminus as *LEPRb*, but different C termini. Except for the hypothalamus, these shorter forms are found in much higher abundance in all other *LEPR*-expressing tissues. The role of the short forms of the receptor in leptin signaling is minor and their major effect is in modifying circulating leptin activity, bioavailability, and transport through the blood–brain barrier [98,111–114]. A few studies have demonstrated the presence of short forms of *LEPR* mRNA in avian species. In chickens, Liu *et al.* [48] detected a short *LEPR* transcript lacking the last exon, similar to the most abundantly expressed variant in mammals (*LEPRa* also known as *OB-Ra* [113]). However, the chicken *LEPRa*-like transcript was expressed at low levels and was absent from the choroid plexus, where in mammals, *LEPRa* expression is at its highest, assisting transfer of leptin through the blood–brain barrier [48]. In quail, Wang *et al.* [25] characterized three alternatively spliced *LEPR* mRNAs, which contained the 5′ end of *LEPR* mRNA but lacked its predicted transmembrane domain and were therefore assumed to encode soluble forms of LEPR. Among these isoforms, only one (*LEPRc*) was detected at significant mRNA levels in the pituitary and kidney of quails [25]. However, since this was shown only by PCR amplification, additional support is needed such as by full-length mRNA sequencing [115] and/or identification of soluble LEPR in the blood circulation of chicken/avian species. The dominant expression of full-length *LEPR* in chicken tissues was demonstrated by comparative profiling with available GenBank RNA-Seq data using as bait, *LEPR* sequences that are common to all of the known *LEPR* transcripts versus the sequence of the last exon, which is only present in the long form of *LEPR* [6]. Therefore, it seems that in Aves the predominantly expressed *LEPR* isoform is the long form in all tissues [6,48]. Since the short forms of LEPR in mammals are implicated in clearance, transport, and stabilization of circulating leptin, this result supports the suggestion that in avian species, blood-circulating leptin is not significant and leptin operates locally in autocrine/paracrine fashion.

action. Paracrine/autocrine mode of leptin action is also supported by the lack of significant expression of short forms of *LEPR* in birds (Box 3). Nevertheless, an exceptionally high level of *LEPR* mRNA is found in the pituitary of chicken, rock dove quail, and zebra finch [2,4,6,25], more than 100-fold higher than *LEPR* in any other tissues (Figure 2) [2,4,6,48]. This raises the possibility that pituitary LEPR responds to leptin in the cerebral fluid or in the median eminence of the hypothalamus, thus retaining some of the endocrine role of leptin. However, it is also possible that the high *LEPR* mRNA in the pituitary evolved to maximize the response to the pituitary leptin. Given that in mammals *LEPRb* is expressed at a much higher level in the hypothalamus compared to pituitary [26,32], the dominant level of avian *LEPR* expression in the pituitary suggests its implication in regulation of reproduction and stress response rather than appetite. In other nonmammalian vertebrates, such as fish and frogs, *LEPR* is also expressed at a high level in the pituitary, albeit not higher than in the hypothalamus [49,50]. In frogs, fish, and lizards, leptin has been shown to stimulate the production of the reproduction-related pituitary hormones prolactin and follicle-stimulating hormone [51–53], similar to the role of leptin in the pituitary of mammals [54]. However, in teleost fish, RNA-Seq analysis of pituitary explants following incubation with leptin indicated strong induction of the glycolysis circuit rather than of genes related to control of reproduction [55]. Therefore, expression of *LEPR* in the pituitary is common in all vertebrates and peaks in birds but its exact role awaits further study.

Leptin in Avian Blood Circulation

Consistent with the low leptin expression observed at the mRNA level, leptin activity in the blood circulation of birds is usually undetectable by the chicken LEPR-based bioassays in cell culture [28]. The sensitivity of this assay allows detection of leptin in serum samples of obese and lean humans and cows, which crossreact with the chicken LEPR [6,28] but not in serum samples from commercial chicken lines, red jungle fowls, geese, and quails under a variety of physiological conditions. Moreover, leptin activity was absent in blood samples collected from birds with strong seasonal variation in voluntary food intake and fat accumulation. These include samples from preincubation and chick-rearing periods of Ad lie penguins (*Pygoscelis adeliae*), and samples from bar-tailed godwits obtained during migratory flight and refueling stages [56]. In contrast, leptin was observed in the blood circulation of three out of ten rock doves, obtained from a service company that captures city doves

[4]. These doves also had high liver leptin expression (Box 2), which may be the source of the rare indication of circulating leptin. Other reports showing leptin activity in blood samples of birds [34,57–59] used nonverified assay systems, and therefore await further verification (Box 1).

In summary, it appears that avian leptin expression is operating at low levels in an autocrine/paracrine fashion, and is usually undetectable in circulation. It is possible that the sporadic cases of relatively high leptin expression levels in the liver (Box 2) lead to a more significant amount of leptin in the circulation. However, at least in doves, rare incidences of blood leptin activity was found in sexually mature males with high body weight and low fat stores living in the city where they may have been exposed to pathogenic infection, or extreme physiological conditions [4].

Role of Avian Leptin

The delay in the discovery of genuine chicken leptin and the lack of a simple method to produce transgenic chicken lines are the primary reasons for the lack of knowledge about the role of leptin in birds. During the two decades between the discovery of mammalian and bird leptins, over 100 papers were published based on an erroneous leptin sequence. Some of these papers are clearly misleading, such as those using the erroneous leptin sequences/peptides as a probe for hybridization, PCR amplification or establishing radioimmunoassays [58–62]. These reports showed leptin expression in adipose, liver, and blood samples, usually in correlation with energy availability, and provided results that contradicted those obtained using genuine molecular tools [2,4,6,11].

Because vertebrate leptins have a conserved 3D structure [9,28,35,36,63] allowing for heterologous LEPRs activation *in vitro*, studies involving administration of heterologous leptins in Aves were assumed to represent true leptin signaling. However, reports on leptin administration to birds are largely controversial. Several studies showed that leptin attenuates food intake upon its intracerebroventricular or peripheral administration in chickens, great tits (*Parus major*), and Asian blue quail (*Coturnix chinensis*) [64–69]. In contrast, other reports found no effect of leptin administration on food intake, enhanced body weight, or food consumption [70–72]. In addition, treatment of leghorn chickens with a potent leptin antagonist that blocks chicken LEPR signaling *in vitro* had no effect on food intake or body weight during 10 days of daily injection [73]. Similar treatment with the same antagonist in rodents dramatically enhanced food consumption and body weight from the first day of treatment [74].

Other studies of heterologous leptin administration to birds pointed to effects of leptin on embryonic development (Box 4), and enhancement of puberty and reproduction in chicken and great tits [72,75], whereas in Asian blue quail, leptin treatment hampered male reproduction [68]. Other reported effects of leptin administration include enhancing markers of autophagy in the hypothalamus and liver

Box 4. Leptin in Embryos

Mice and humans with null mutations in leptin or *LEPR* are born normal [116,117]. This indicates that leptin is not essential for embryonal development in these species. However, since both leptin- and *LEPR*-deficient subjects are sterile and can be produced only by heterozygous parents, maternal leptin and LEPR in extraembryonic tissues may operate during embryogenesis, as is known for the process of implantation [118,119]. Moreover, since leptin is produced by the placenta in significant amounts, with no relation to obesity [120,121], it is possible that minor developmental roles of leptin are not noticed in the mutant offspring due to the dominant phenotype of the null mutations manifested immediately after birth. In nonmammalian vertebrates, some reports attributed a role for leptin in embryonic development. For example, in frogs, administration or blocking of leptin during early prometamorphosis affected growth and development of the hind limb [9]. In Aves, *in ovo* leptin administration has produced controversial results. In some experiments, leptin treatment enhanced embryo growth and development and even growth after hatching [122–124], whereas in another report leptin administration did the opposite [124]. Similarly, the effect of leptin on the vasculature of the chorioallantoic membrane was inhibitory in the latter report, but stimulatory in another [125]. In chick embryos, leptin mRNA was observed in limb buds by *in situ* hybridization [6]. However, direct proof of the role of leptin in chick development, such as by producing congenital loss of leptin or *LEPR*, is still missing.

[69], attenuating testosterone-induced immunosuppression [76], and reducing plasma cholesterol and triglycerides [68].

Taken together, these studies of leptin administration to birds do not provide a clear understanding of its role. It is possible that *in vivo*, heterologous leptins elicit different responses than endogenous leptins even though their 3D structure is similar. Since it is clear that avian leptin is not the signal of energy stored in the adipose tissue, it is likely that the noncoherent reports concerning its effect on food intake and body weight point to no such effect. The reports linking leptin activity with reproduction are compatible with the expression of avian leptin and *LEPR* in the tissues implicated in the hypothalamic–pituitary–gonadal and –adrenal axes. However, further investigation is needed to establish this possibility.

Leptin in Nonplacental Nonavian Vertebrates

Nonmammalian leptins were first found in a number of teleost fish, and frogs [8–10]. These leptins displayed low sequence similarity to mammalian leptins (13–30% amino acid identity with humans) and low sequence similarity among them (30–50% amino acid identity depending on evolutionary relatedness). Genuine leptins of nonmammalian vertebrates as well as those identified later on had the predicted protein structures and local synteny at the genomic mapping positions that were similar to mammalian leptins [8–10,38,77]. Like mammals, tetrapods generally possess one leptin gene, except for frogs and some lizards, which have two leptin orthologs. These duplications are thought to have evolved ~34 mya by allotetraploidy in frogs (Figure 3, leptin s and l; [78]) and through a recombination event that includes leptin A and the neighboring *RBM28* gene in lizards [35] (Figure 3). Teleost fish have two leptin genes (A and B), or four in salmonid and cyprinid lineages. Based on a consideration of local synteny, polygenic analyses, and evolutionary time calculations, it is thought that the two teleostan leptin genes were produced by the third teleost-specific whole-genome duplication event (Figure 3; leptin A and B), and the four leptins in salmonid and cyprinid lineages resulted from additional lineage-specific genome duplications [79,80]. In most teleost fish, the expression level of leptin mRNA is highest in the liver and low or absent in the adipose tissue [81]. Additional sites of leptin expression in teleost fish are the brain, gonads, muscles, and kidneys, with significant variation among species. *LEPR* is prominently expressed in the pituitary, hypothalamus, and gonads. After numerous reports linking fish leptin to the control of appetite and reproduction as reviewed by Copeland *et al.*, [82], a knockout study on *LEPR* and leptin A in zebrafish [83] showed that leptin is not implicated in adipostasis, appetite, or reproduction control but rather in the regulation of beta cells development and activity. This clear demonstration is also supported by a study in European eels, showing that 4-month fasting had no impact on the expression of leptins or *LEPR* [79]. In contrast, frog leptin has been shown to stimulate development of tadpole limbs [9] and lungs [84], and to appear in the adipose tissue (fat body) and to a lesser extent in the liver after metamorphosis, when *LEPR* is expressed in the preoptic area/hypothalamus and pituitary [85]. Furthermore, administration of recombinant frog leptin after metamorphosis suppresses appetite [50]. This administration upregulates *SOCS3*, *POMC*, and *c-fos* mRNAs in the preoptic area/hypothalamus/pituitary region of the frog similar to its effect in mammals [53]. However, in another amphibian species, tiger salamander (*Ambystoma tigrinum*), expression of leptin mRNA was detected only in male testes, and in females, it was dominant in skin and brain [10]. In lizards, leptin has been implicated in glucoregulation and the regulation of reproduction instead of adipostat activity by some authors [86–88], and in ameliorating immunity, body temperature, and satiety by others [89,90]. In turtle (*Eretmochelys imbricata*), low blood leptin was correlated with prolonged fasting [91]. In marsupials, leptin increases energy expenditure [92], and inhibits body weight and food intake [93,94]. However, in a representative of the monotreme marsupial clade, which are egg laying and more closely related to reptiles [95], plasma leptin correlates with the reproductive cycle and not with body mass [96].

In conclusion, conflicting reports about the role of leptin in nonmammalian vertebrates are not confined to birds but also present in studies of other vertebrate species. The discrepancies in these studies may result in part from the use of heterologous leptins and nonverified molecular tools, such as heterologous nucleic acid probes and antibodies. For both of these reasons, production of *LEPR*

knockout lines using CRISPR cas9 technology will be of importance. So far, this has been used among nonmammalian vertebrates in zebrafish [97], disputing earlier suggestions of adipostat role of leptin in teleost fish [82].

Concluding Remarks and Future Perspectives

The avian GC-rich leptins are highly diverged genes, both among bird species and comparing to other vertebrate clades. The pattern of expression of bird leptins also differs greatly from that of mammals, suggesting a different role and mode of action (see Outstanding Questions). The apparent autocrine/paracrine instead of endocrine characteristic of leptin in birds is based on the observation of cotranscription of leptin and *LEPR*, usually undetectable levels of blood leptin, and lack of the short forms of *LEPR*, which are known to mediate its blood clearance and to modulate its activity and transfer through the blood–brain barrier [98]. In addition, the surprising and unexpected finding that leptin is missing from the adipose tissue, observed under variety of physiological and feeding conditions, indicates that in birds, leptin is not the signal by which the adipose tissue announces the amount of fat stores. This finding is compatible with the switch of the dominant site of mRNA expression of *LEPR* in Aves from the pituitary to the hypothalamus in mammals. It seems that since leptin in Aves is not the adipokine that signals the amount of fat stores, it is not implicated in the hypothalamic control of appetite. This finding raises the question of whether another adipokine replaces leptin in the bird adipose tissue, or whether this tissue has a different endocrine role. The latter possibility was recently supported by showing a shift in the endocrine role of visceral fat of chickens toward reproduction [40]. Moreover, the same study revealed that in addition to leptin, other related adipokines (TNF, IL6, and IFG), implicated in the control of appetite, insulin resistance and inflammation in mammals [99], do not function as adipokines in chicken [40]. This hypothesis, based on mRNA-expression profiles, corroborates the 30-year-old observation made from physiological characterization that obesity and insulin resistance are dissociated in chickens [44,46]. In birds, the ability to fly and migrate seems to alleviate the threat of starvation. Thus, unrestrained accumulation of fat reserves before migration, wintering or chick rearing, which can reach 120% of lean body mass [100], seems more critical for survival than adipostat control.

In summary, despite highly controversial results, a critical review of the published data reveals that leptin in Aves does not operate as an adipostat. Moreover, additional adipokines with inflammatory activity in mammals do not seem to operate as adipokines in Aves, altogether suggesting a different endocrine role for bird versus mammalian adipose tissue. It seems that the robust adipostat role of leptin has developed during the evolution of mammals, working in concert with other adipokines and dramatically affecting the endocrine role of the adipose tissue. Nevertheless, we cannot exclude the possibility that the adipostat role of leptin has emerged in an early common ancestor of mammals and other vertebrate clades as has been suggested for frogs [53] and adapted diverged functions during the evolution of descending clades (Figure 3).

Outstanding Questions

What is the role of leptin in birds? Despite the technical difficulty in obtaining transgenic chickens, producing lines of chickens with leptin or *LEPR* gene knockouts should be considered as the ultimate methodology to solve this question.

What is the role of the sporadic cases of leptin expression in the liver? Why is this expression level much higher than leptin mRNA expression in other tissues (>4–10 RPKM versus <0.5 RPKM, respectively). Understanding these rare cases of high leptin expression in the liver may shed light on the role of leptin in birds.

How is feed intake controlled in birds? Is it mainly restricted mechanically by the capacity of the crop and/or by signals from the digestive system, such as ghrelin in the proventriculus and secretin in the duodenum, that act as satiety/hunger signals?

Why does adiposity convey inflammation and insulin resistance in mammals and not in birds? Such a study might reveal the evolutionary force behind the mechanism leading to the metabolic syndrome in humans.

Is the reproductive role of the mammalian leptin in the pituitary conserved in all vertebrates expressing *LEPR*, albeit the apparent variability in its role as adipostat?

Besides stabilizing chromosome pairing during meiosis, is there a biologically selective benefit for some genes, such as leptin, to have high GC content?

Acknowledgments

We thank Leif Andersson, Sara Yosefi, Guy Horev, Gideon Hen, Anna Ronin, Nataly Novoseletsky, Anna Molochnikov, Susanne Bornelöv, Sharon Benjamini, Andrey Shirak, Shoval Mayra, Saibaba Ganesan, and Maxim Simantov for their valuable discussion, and Israel Science Foundation (ISF), Israel (Grant 1294/17).

References

1. Zhang, Y. *et al.* (1994) Positional cloning of the mouse obese gene and its human homologue. *Nature* 372, 425–432
2. Huang, G. *et al.* (2014) Discovery of a novel functional leptin protein (LEP) in zebra finches: evidence for the existence of an authentic avian leptin gene predominantly expressed in the brain and pituitary. *Endocrinology* 155, 3385–3396
3. Friedman-Einat, M. and Seroussi, E. (2014) Quack leptin. *BMC Genomics* 15, 551
4. Friedman-Einat, M. *et al.* (2014) Discovery and characterization of the first genuine avian leptin gene in the rock dove (*Columba livia*). *Endocrinology* 155, 3376–3384
5. Prokop, J.W. *et al.* (2014) Discovery of the elusive leptin in birds: identification of several 'missing links' in the evolution of leptin and its receptor. *PLoS One* 9, e92751
6. Seroussi, E. *et al.* (2016) Identification of the long-sought leptin in chicken and duck: expression

pattern of the highly GC-rich avian leptin fits an autocrine/paracrine rather than endocrine function. *Endocrinology* 157, 737–751
7. Seroussi, E. *et al.* (2017) Mapping of leptin and its syntenic genes to chicken chromosome 1p. *BMC Genet.* 18, 77
8. Kurokawa, T. *et al.* (2005) Identification of cDNA coding for a homologue to mammalian leptin from pufferfish, *Takifugu rubripes*. *Peptides* 26, 745–750
9. Crespi, E.J. and Denver, R.J. (2006) Leptin (ob gene) of the South African clawed frog *Xenopus laevis*. *Proc. Natl. Acad. Sci. U. S. A.* 103, 10092–10097
10. Boswell, T. *et al.* (2006) Identification of a non-mammalian leptin-like gene: characterization and expression in the tiger salamander (*Ambystoma tigrinum*). *Gen. Comp. Endocrinol.* 146, 157–166
11. Farkašová, H. *et al.* (2016) Identification of a GC-rich leptin gene in chicken. *Agri Gene* 1, 88–92
12. Jarvis, E.D. *et al.* (2014) Whole-genome analyses resolve early branches in the tree of life of modern birds. *Science* 346, 1320–1331
13. Burt, D.W. (2006) The chicken genome. *Genome Dyn.* 2, 123–137
14. Hron, T. *et al.* (2015) Hidden genes in birds. *Genome Biol.* 16, 164
15. Denyer, M.P. *et al.* (2016) Missed, not missing: phylogenomic evidence for the existence of avian FoxP3. *PLoS One* 11, e0150988
16. Bornelov, S. *et al.* (2017) Correspondence on Lovell *et al.*: identification of chicken genes previously assumed to be evolutionarily lost. *Genome Biol.* 18, 112
17. Yin, Z.T. *et al.* (2019) Revisiting avian 'missing' genes from *de novo* assembled transcripts. *BMC Genomics* 20, 4
18. Lovell, P.V. *et al.* (2014) Conserved syntenic clusters of protein coding genes are missing in birds. *Genome Biol.* 15, 565
19. Zhang, G. *et al.* (2014) Comparative genomics reveals insights into avian genome evolution and adaptation. *Science* 346, 1311–1320
20. Horev, G. *et al.* (2000) Molecular cloning and properties of the chicken leptin-receptor (CLEPR) gene. *Mol. Cell. Endocrinol.* 162, 95–106
21. Ohkubo, T. *et al.* (2000) Structure and tissue distribution of chicken leptin receptor (cOb-R) mRNA. *Biochim. Biophys. Acta* 1491, 303–308
22. Dunn, I.C. *et al.* (2000) Mapping of the leptin receptor gene (LEPR) to chicken chromosome 8. *Anim. Genet.* 31, 290
23. Richards, M.P. and Poch, S.M. (2003) Molecular cloning and expression of the turkey leptin receptor gene. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 136, 833–847
24. Wang, F. *et al.* (2011) Molecular cloning, expression, and regulation of goose leptin receptor gene in adipocytes. *Mol. Cell. Biochem.* 353, 267–274
25. Wang, D. *et al.* (2016) Discovery and functional characterization of leptin and its receptors in Japanese quail (*Coturnix japonica*). *Gen. Comp. Endocrinol.* 225, 1–12
26. Tartaglia, L.A. (1997) The leptin receptor. *J. Biol. Chem.* 272, 6093–6096
27. Fong, T.M. *et al.* (1998) Localization of leptin binding domain in the leptin receptor. *Mol. Pharmacol.* 53, 234–240
28. Hen, G. *et al.* (2008) Monitoring leptin activity using the chicken leptin receptor. *J. Endocrinol.* 197, 325–333
29. Adachi, H. *et al.* (2008) Chicken leptin receptor is functional in activating JAK-STATpathway *in vitro*. *J. Endocrinol.* 197, 335–342
30. Niv-Spector, L. *et al.* (2005) Mapping leptin-interacting sites in recombinant leptin-binding domain (LBD) subcloned from chicken leptin receptor. *Biochem. J.* 390, 475–484
31. Wauman, J. and Tavernier, J. (2011) Leptin receptor signaling: pathways to leptin resistance. *Front. Biosci. (Landmark Ed)* 16, 2771–2793
32. Friedman, J.M. (1997) Leptin, leptin receptors and the control of body weight. *Eur. J. Med. Res.* 2, 7–13
33. Pedroso, J.A. *et al.* (2016) Changes in leptin signaling by SOCS3 modulate fasting-induced hyperphagia and weight regain in mice. *Endocrinology* 157, 3901–3914
34. Ohkubo, T. *et al.* (2014) Avian blood induced intranuclear translocation of STAT3 via the chicken leptin receptor. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 174, 9–14
35. Denver, R.J. *et al.* (2011) Evolution of leptin structure and function. *Neuroendocrinology* 94, 21–38
36. Prokop, J.W. *et al.* (2012) Leptin and leptin receptor: analysis of a structure to function relationship in interaction and evolution from humans to fish. *Peptides* 38, 326–336
37. Hammond, J.A. *et al.* (2012) Phocid seal leptin: tertiary structure and hydrophobic receptor binding site preservation during distinct leptin gene evolution. *PLoS One* 7, e35395
38. Hammond, J.A. *et al.* (2005) Molecular cloning and expression of leptin in gray and harbor seal blubber, bone marrow, and lung and its potential role in marine mammal respiratory physiology. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 289, R545–R553
39. Yang, J. *et al.* (2011) Functional evolution of leptin of *Ochotona curzoniae* in adaptive thermogenesis driven by cold environmental stress. *PLoS One* 6, e19833
40. Bornelov, S. *et al.* (2018) Comparative omics and feeding manipulations in chicken indicate a shift of the endocrine role of visceral fat towards reproduction. *BMC Genomics* 19, 295
41. Resnyk, C.W. *et al.* (2017) Transcriptional analysis of abdominal fat in chickens divergently selected on bodyweight at two ages reveals novel mechanisms controlling adiposity: validating visceral adipose tissue as a dynamic endocrine and metabolic organ. *BMC Genomics* 18, 626
42. Resnyk, C.W. *et al.* (2015) RNA-seq analysis of abdominal fat in genetically fat and lean chickens highlights a divergence in expression of genes controlling adiposity, hemostasis, and lipid metabolism. *PLoS One* 10, e0139549
43. Touchburn, S. *et al.* (1981) Evidence of a glucose-insulin imbalance and effect of dietary protein and energy level in chickens selected for high abdominal fat content. *J. Nutr.* 111, 325–335
44. Simon, J. and Leclercq, B. (1982) Longitudinal study of adiposity in chickens selected for high or low abdominal fat content: further evidence of a glucose-insulin imbalance in the fat line. *J. Nutr.* 112, 1961–1973
45. Kahn, S.E. *et al.* (2006) Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature* 444, 840–846
46. Leclercq, B. *et al.* (1987) Effects of selection for high and low plasma glucose concentration in chickens. *Br. Poult. Sci.* 28, 557–566
47. Friedman, J. (2016) The long road to leptin. *J. Clin. Invest.* 126, 4727–4734
48. Liu, X. *et al.* (2007) Molecular cloning and tissue distribution of a short form chicken leptin receptor mRNA. *Domest. Anim. Endocrinol.* 32, 155–166
49. Yan, A. *et al.* (2017) Leptin stimulates prolactin mRNA expression in the goldfish pituitary through a combination of the PI3K/Akt/mTOR, $MKK_{3/6}$/p(38) MAPK and $MEK_{1/2}$/$ERK_{1/2}$ signalling pathways. *Int. J. Mol. Sci.* 18, E2781

50. Bender, M.C. *et al.* (2018) To eat or not to eat: ontogeny of hypothalamic feeding controls and a role for leptin in modulating life-history transition in amphibian tadpoles. *Proc. Biol. Sci.* 285, 20172784
51. Tipsmark, C.K. *et al.* (2008) Leptin stimulates pituitary prolactin release through an extracellular signal-regulated kinase-dependent pathway. *J. Endocrinol.* 196, 275–281
52. Ferrandino, I. *et al.* (2015) Effects of leptin on FSH cells in the pituitary gland of *Podarcis siculus*. *C. R. Biol.* 338, 180–184
53. Cui, M.Y. *et al.* (2014) Ancient origins and evolutionary conservation of intracellular and neural signaling pathways engaged by the leptin receptor. *Endocrinology* 155, 4202–4214
54. Yu, W.H. *et al.* (1997) Nitric oxide mediates leptin-induced luteinizing hormone-releasing hormone (LHRH) and LHRH and leptin-induced LH release from the pituitary gland. *Endocrinology* 138, 5055–5058
55. Douros, J.D. *et al.* (2018) Leptin stimulates cellular glycolysis through a STAT3 dependent mechanism in *Tilapia*. *Front. Endocrinol. (Lausanne)* 9, 465
56. Yosefi, S. *et al.* (2010) Lack of leptin activity in blood samples of Adelie penguin and bar-tailed godwit. *J. Endocrinol.* 207, 113–122
57. Dridi, S. *et al.* (2000) A chicken leptin-specific radioimmunoassay. *Domest. Anim. Endocrinol.* 18, 325–335
58. Simon, J. *et al.* (2011) Plasma insulin levels are rather similar in chicken and rat. *Gen. Comp. Endocrinol.* 171, 267–268
59. Hennin, H.L. *et al.* (2019) Plasma mammalian leptin analogue predicts reproductive phenology, but not reproductive output in a capital-income breeding seaduck. *Ecol. Evol.* 9, 1512–1522
60. Cassy, S. *et al.* (2003) Leptin and insulin downregulate leptin receptor gene expression in chicken-derived leghorn male hepatoma cells. *Poult. Sci.* 82, 1573–1579
61. Dridi, S. *et al.* (2005) Potential role of leptin in increase of fatty acid synthase gene expression in chicken liver. *Domest. Anim. Endocrinol.* 29, 646–660
62. Lohmus, M. *et al.* (2011) Effects of chronic leptin administration on nitric oxide production and immune responsiveness of greenfinches. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 158, 560–565
63. Londraville, R.L. *et al.* (2017) On the molecular evolution of leptin, leptin receptor, and endospanin. *Front. Endocrinol. (Lausanne)* 8, 58
64. Denbow, D.M. *et al.* (2000) Leptin-induced decrease in food intake in chickens. *Physiol. Behav.* 69, 359–362
65. Dridi, S. *et al.* (2000) Biological activities of recombinant chicken leptin C4S analog compared with unmodified leptins. *Am. J. Physiol. Endocrinol. Metab.* 279, E116–E123
66. Lohmus, M. *et al.* (2003) Leptin depresses food intake in great tits (*Parus major*). *Gen. Comp. Endocrinol.* 131, 57–61
67. Dridi, S. *et al.* (2005) Mode of leptin action in chicken hypothalamus. *Brain Res.* 1047, 214–223
68. Lohmus, M. *et al.* (2006) Chronic administration of leptin in Asian Blue Quail. *J. Exp. Zool. A Comp. Exp. Biol.* 305, 13–22
69. Piekarski, A. *et al.* (2018) AMP-activated protein kinase mediates the effect of leptin on avian autophagy in a tissue-specific manner. *Front. Physiol.* 9, 541
70. Bungo, T. *et al.* (1999) Intracerebroventricular administration of mouse leptin does not reduce food intake in the chicken. *Brain Res.* 817, 196–198
71. Sims, W. *et al.* (2017) Central injection of a synthetic chicken partial leptin peptide does not affect food intake in chicks. *Neurosci. Lett.* 656, 165–168
72. Paczoska-Eliasiewicz, H.E. *et al.* (2006) Exogenous leptin advances puberty in domestic hen. *Domest. Anim. Endocrinol.* 31, 211–226
73. Gertler, A. *et al.* (2014) Pegylated leptin antagonist with strong orexigenic activity in mice is not effective in chickens. *J. Exp. Biol.* 217, 180–184
74. Elinav, E. *et al.* (2009) Pegylated leptin antagonist is a potent orexigenic agent: preparation and mechanism of activity. *Endocrinology* 150, 3083–3091
75. Lohmus, M. and Bjorklund, M. (2009) Leptin affects life history decisions in a passerine bird: a field experiment. *PLoS One* 4, e4602
76. Alonso-Alvarez, C. *et al.* (2007) Energetic reserves, leptin and testosterone: a refinement of the immunocompetence handicap hypothesis. *Biol. Lett.* 3, 271–274
77. Gorissen, M. *et al.* (2009) Two divergent leptin paralogues in zebrafish (*Danio rerio*) that originate early in teleostean evolution. *J. Endocrinol.* 201, 329–339
78. Session, A.M. *et al.* (2016) Genome evolution in the allotetraploid frog *Xenopus laevis*. *Nature* 538, 336–343
79. Morini, M. *et al.* (2015) Duplicated leptin receptors in two species of eel bring new insights into the evolution of the leptin system in vertebrates. *PLoS One* 10, e0126008
80. Berthelot, C. *et al.* (2014) The rainbow trout genome provides novel insights into evolution after whole-genome duplication in vertebrates. *Nat. Commun.* 5, 3657
81. Deck, C.A. *et al.* (2017) Assessing the functional role of leptin in energy homeostasis and the stress response in vertebrates. *Front. Endocrinol. (Lausanne)* 8, 63
82. Copeland, D.L. *et al.* (2011) Leptin in teleost fishes: an argument for comparative study. *Front. Physiol.* 2, 26
83. Federico, C. *et al.* (2005) Avian genomes: different karyotypes but a similar distribution of the GC-richest chromosome regions at interphase. *Chromosom. Res.* 13, 785–793
84. Torday, J.S. *et al.* (2009) Leptin stimulates *Xenopus* lung development: evolution in a dish. *Evol. Dev.* 11, 219–224
85. Bender, M.C. *et al.* (2017) Leptin induces mitosis and activates the canonical Wnt/beta-catenin signaling pathway in neurogenic regions of *Xenopus* tadpole brain. *Front. Endocrinol. (Lausanne)* 8, 99
86. Putti, R. *et al.* (2009) Leptin effects on testis and epididymis in the lizard *Podarcis sicula*, during summer regression. *Gen. Comp. Endocrinol.* 160, 168–175
87. Paolucci, M. *et al.* (2006) Effects of leptin administration on the endocrine pancreas and liver in the lizard *Podarcis sicula*. *J. Exp. Zool. A Comp. Exp. Biol.* 305, 383–395
88. Spanovich, S. *et al.* (2006) Seasonal effects on circulating leptin in the lizard *Sceloporus undulatus* from two populations. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 143, 507–513
89. Wang, A.Z. *et al.* (2019) Leptin ameliorates the immunity, but not reproduction, trade-off with endurance in lizards. *J. Comp. Physiol. B.* 189, 261–269
90. Niewiarowski, P.H. *et al.* (2000) Phenotypic effects of leptin in an ectotherm: a new tool to study the evolution of life histories and endothermy? *J. Exp. Biol.* 203, 295–300
91. Goldberg, D.W. *et al.* (2013) Ghrelin and leptin modulate the feeding behaviour of the hawksbill

turtle *Eretmochelys imbricata* during nesting season. *Conserv. Physiol.* 1, cot016
92. Geiser, F. *et al.* (1998) Leptin increases energy expenditure of a marsupial by inhibition of daily torpor. *Am. J. Phys.* 275, R1627–R1632
93. Wittert, G.A. *et al.* (2004) Leptin prevents obesity induced by a high-fat diet after diet-induced weight loss in the marsupial *S. crassicaudata*. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 286, R734–R739
94. Hope, P.J. *et al.* (1999) Effect of diet on the response to leptin in the marsupial *Sminthopsis crassicaudata*. *Am. J. Phys.* 276, R373–R381
95. Nicol, S.C. (2017) Energy homeostasis in monotremes. *Front. Neurosci.* 11, 195
96. Sprent, J. *et al.* (2012) Does leptin signal adiposity in the egg-laying mammal, *Tachyglossus aculeatus*? *Gen. Comp. Endocrinol.* 178, 372–379
97. Michel, M. *et al.* (2016) Leptin signaling regulates glucose homeostasis, but not adipostasis, in the zebrafish. *Proc. Natl. Acad. Sci. U. S. A.* 113, 3084–3089
98. Hileman, S.M. *et al.* (2002) Characterizaton of short isoforms of the leptin receptor in rat cerebral microvessels and of brain uptake of leptin in mouse models of obesity. *Endocrinology* 143, 775–783
99. Ahima, R.S. and Lazar, M.A. (2008) Adipokines and the peripheral and neural control of energy balance. *Mol. Endocrinol.* 22, 1023–1031
100. Bayly, N.J. (2007) Extreme fattening by sedge warblers, *Acrocephalus schoenobaenus*, is not triggered by food availability alone. *Anim. Behav.* 74, 471–479
101. Sereno, P.C. (1999) The evolution of dinosaurs. *Science* 284, 2137–2147
102. Hedges, S.B. (2002) The origin and evolution of model organisms. *Nat. Rev. Genet.* 3, 838–849
103. Reisz, R.R. and Muller, J. (2004) Molecular timescales and the fossil record: a paleontological perspective. *Trends Genet.* 20, 237–241
104. Wagner, G.P. *et al.* (2012) Measurement of mRNA abundance using RNA-seq data: RPKM measure is inconsistent among samples. *Theory Biosci.* 131, 281–285
105. Zeferino, C.P. *et al.* (2017) Changes in renal gene expression associated with induced ochratoxicosis in chickens: activation and deactivation of transcripts after varying durations of exposure. *Poult. Sci.* 96, 1855–1865
106. Taouis, M. *et al.* (1998) Cloning the chicken leptin gene. *Gene* 208, 239–242
107. Ashwell, C.M. *et al.* (1999) Hormonal regulation of leptin expression in broiler chickens. *Am. J. Phys.* 276, R226–R232
108. Piche, T. *et al.* (2004) The severity of liver fibrosis is associated with high leptin levels in chronic hepatitis C. *J. Viral Hepat.* 11, 91–96
109. Romero-Gomez, M. *et al.* (2003) Serum leptin levels correlate with hepatic steatosis in chronic hepatitis C. *Am. J. Gastroenterol.* 98, 1135–1141
110. Comlekci, A. *et al.* (2003) Serum leptin levels in patients with liver cirrhosis and chronic viral hepatitis. *Scand. J. Gastroenterol.* 38, 779–786
111. Lee, G.H. *et al.* (1996) Abnormal splicing of the leptin receptor in diabetic mice. *Nature* 379, 632–635
112. Barr, V.A. *et al.* (1999) Subcellular localization and internalization of the four human leptin receptor isoforms. *J. Biol. Chem.* 274, 21416–21424
113. Li, Z. *et al.* (2013) Phenotypic effects of an induced mutation of the ObRa isoform of the leptin receptor. *Mol. Metab.* 2, 364–375
114. Schaab, M. and Kratzsch, J. (2015) The soluble leptin receptor. *Best Pract. Res. Clin. Endocrinol. Metab.* 29, 661–670
115. Anvar, S.Y. *et al.* (2018) Full-length mRNA sequencing uncovers a widespread coupling between transcription initiation and mRNA processing. *Genome Biol.* 19, 46
116. Bray, G.A. *et al.* (1990) Neuroendocrine control of the development of obesity – understanding gained from studies of experimental animal-models. *Front. Neuroendocrinol.* 11, 128–181
117. Farooqi, I.S. *et al.* (2007) Clinical and molecular genetic spectrum of congenital deficiency of the leptin receptor. *N. Engl. J. Med.* 356, 237–247
118. Garcia, M.R. (2017) Leptin contributes to the development of the corpus luteum. *Cell Dev. Biol.* 6, 190
119. Ramos, M.P. *et al.* (2005) Leptin serves as an upstream activator of an obligatory signaling cascade in the embryo-implantation process. *Endocrinology* 146, 694–701
120. Schanton, M. *et al.* (2018) Involvement of leptin in the molecular physiology of the placenta. *Reproduction* 155, R1–R12
121. Masuzaki, H. *et al.* (1997) Nonadipose tissue production of leptin: leptin as a novel placenta-derived hormone in humans. *Nat. Med.* 3, 1029–1033
122. Yuan, L. *et al.* (2017) *In ovo* leptin administration modulates glucocorticoid receptor mRNA expression specifically in the hypothalamus of broiler chickens. *Neurosci. Lett.* 638, 181–188
123. Lamosova, D. *et al.* (2003) Effect of in ovo leptin administration on the development of Japanese quail. *Physiol. Res.* 52, 201–209
124. Su, L. *et al.* (2012) In ovo leptin administration inhibits chorioallantoic membrane angiogenesis in female chicken embryos through the STAT3-mediated vascular endothelial growth factor (VEGF) pathway. *Domest. Anim. Endocrinol.* 43, 26–36
125. Manjunathan, R. and Ragunathan, M. (2015) *In ovo* administration of human recombinant leptin shows dose dependent angiogenic effect on chicken chorioallantoic membrane. *Biol. Res.* 48, 29
126. Zhang, F. *et al.* (1997) Crystal structure of the obese protein leptin-E100. *Nature* 387, 206–209
127. Haglund, E. *et al.* (2012) The unique cysteine knot regulates the pleotropic hormone leptin. *PLoS One* 7, e45654
128. Mohammadzadeh, G. *et al.* (2014) Association of serum soluble leptin receptor and leptin levels with breast cancer. *J. Res. Med. Sci.* 19, 433–438
129. Tamura, K. *et al.* (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol. Biol. Evol.* 30, 2725–2729