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汇报人：邓大鹏

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在罗非鱼中，不同代谢状态下leptin和垂体GH，肝脏GHRs及IGFs的相互调控作用

Control of leptin by metabolic state and its regulatory interactions with pituitary growth hormone and hepatic growth hormone receptors and insulin like growth factors in the tilapia (*Oreochromis mossambicus*)

Jonathan D. Douros [a], David A. Baltzegar [a], Jamie Mankiewicz [a], Jordan Taylor [a], Yoko Yamaguchi [b], Darren T. Lerner [b], Andre P. Seale [d], E. Gordon Grau [b], Jason P. Breves [c], Russell J. Borski [a,*]

[a] Department of Biological Sciences, North Carolina State University, Raleigh, NC 27695-7617, United States
[b] Hawaii Institute of Marine Biology, University of Hawaii, Kaneohe, HI 96744, United States
[c] Department of Biology, Skidmore College, Saratoga Springs, NY 12866, United States
[d] Department of Human Nutrition, Food and Animal Sciences, College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa, Honolulu, HI 96822, United States

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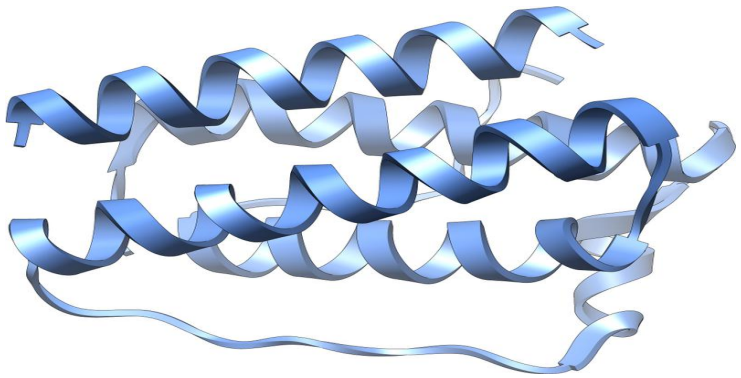
ABSTRACT

Leptin is an important cytokine for regulating energy homeostasis, however, relatively little is known about its function and control in teleost fishes or other ectotherms, particularly with regard to interactions with the growth hormone (GH)/insulin-like growth factors (IGFs) growth regulatory axis. Here we assessed the regulation of LepA, the dominant paralog in tilapia (*Oreochromis mossambicus*) and other teleosts under altered nutritional state, and evaluated how LepA might alter pituitary growth hormone (GH) and hepatic insulin-like growth factors (IGFs) that are known to be disparately regulated by metabolic state. Circulating LepA, and *lepa* and *lepr* gene expression increased after 3-weeks fasting and declined to control levels 10 days following refeeding. This pattern of leptin regulation by metabolic state is similar to that previously observed for pituitary GH and opposite that of hepatic GHR and/or IGF

CONTANTS

01

研究背景



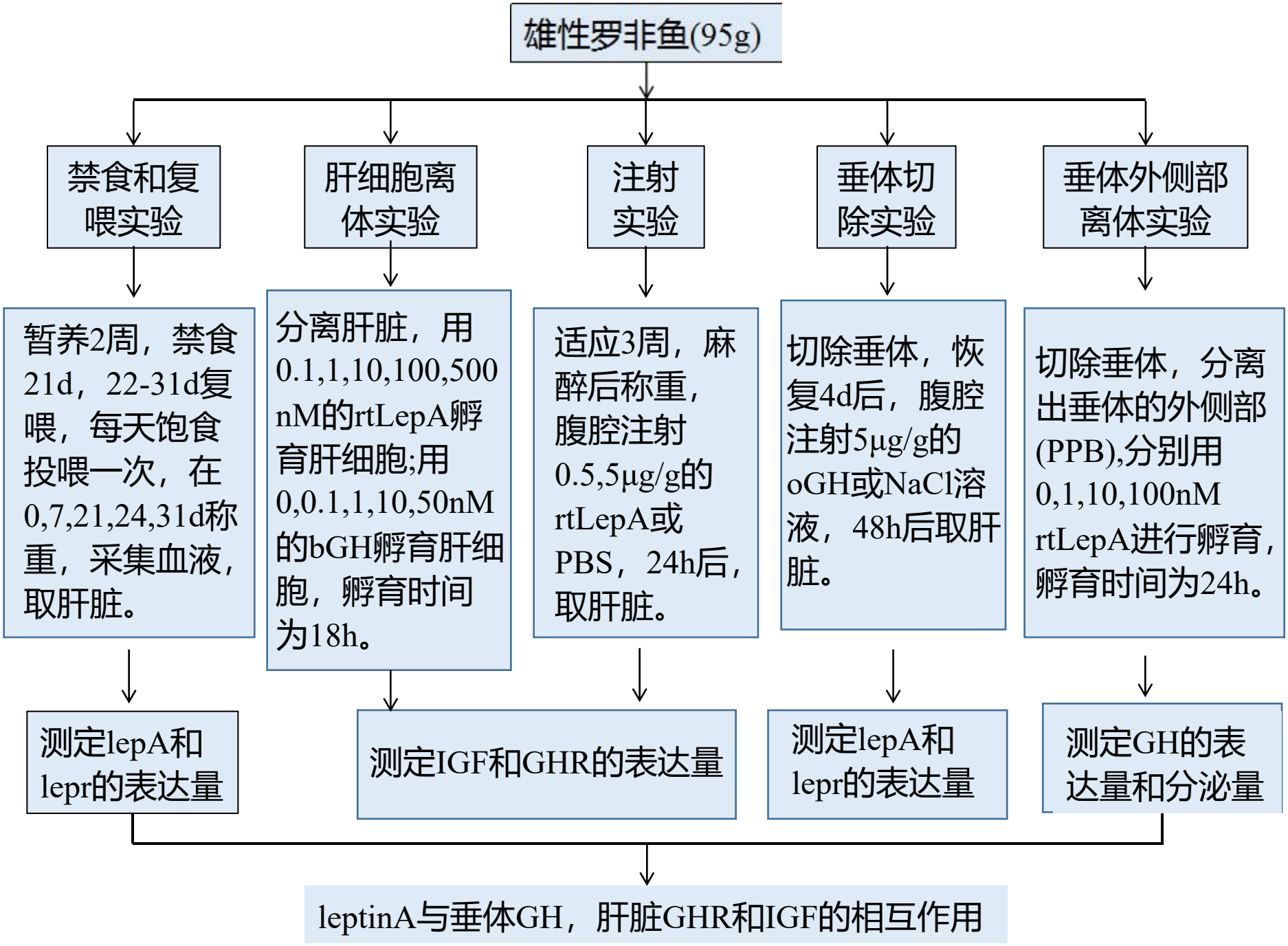
Leptin是一种调节代谢和能量消耗的细胞因子激素，它在生物体中存在形式有多种，在肝脏中它以leptin A的形式存在。与大多数脊椎动物一样，鱼类体细胞的生长主要受内分泌生长激素（GH）/胰岛素样生长因子（IGF）轴的调节。

研究表明，在哺乳动物中，leptin对IGF和GH均有调节作用。在鱼类中，leptin对IGF和GH的调节作用也有诸多研究，但leptin与GH-IGF轴相互作用调节鱼类代谢和生长的机制，还尚不清楚。

本实验以罗非鱼为研究对象，探究不同代谢状态下，leptin对垂体GH，肝脏GHR和IGF的调控作用以及GH本身是否对leptin有调控作用。

02

材料与方法


雄性罗非鱼(95g)
禁食和复喂实验
肝细胞离体实验
注射实验
垂体切除实验
垂体外侧部离体实验
暂养2周，禁食21d，22-31d复喂，每天饱食投喂一次，在0,7,21,24,31d称重，采集血液，取肝脏。
分离肝脏，用0.1,1,10,100,500 nM的rtLepA孵育肝细胞;用0,0.1,1,10,50nM的bGH孵育肝细胞，孵育时间为18h。
适应3周，麻醉后称重，腹腔注射0.5,5μg/g的rtLepA或PBS，24h后，取肝脏。
切除垂体，恢复4d后，腹腔注射5μg/g的oGH或NaCl溶液，48h后取肝脏。
切除垂体，分离出垂体的外侧部(PPB),分别用0,1,10,100nM rtLepA进行孵育，孵育时间为24h。
测定lepA和lepr的表达量
测定IGF和GHR的表达量
测定lepA和lepr的表达量
测定GH的表达量和分泌量
leptinA与垂体GH，肝脏GHR和IGF的相互作用

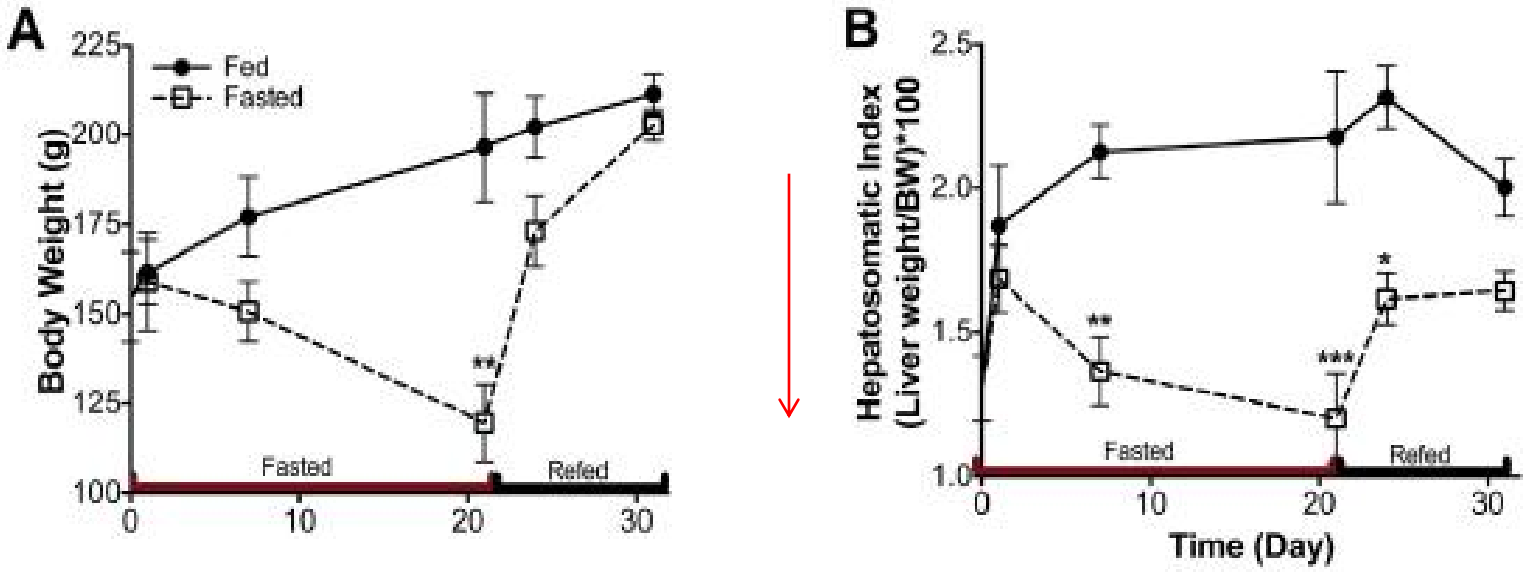
03

结果分析

Table 1
List of PCR primer pairs used for qPCR analysis of various genes in tilapia.

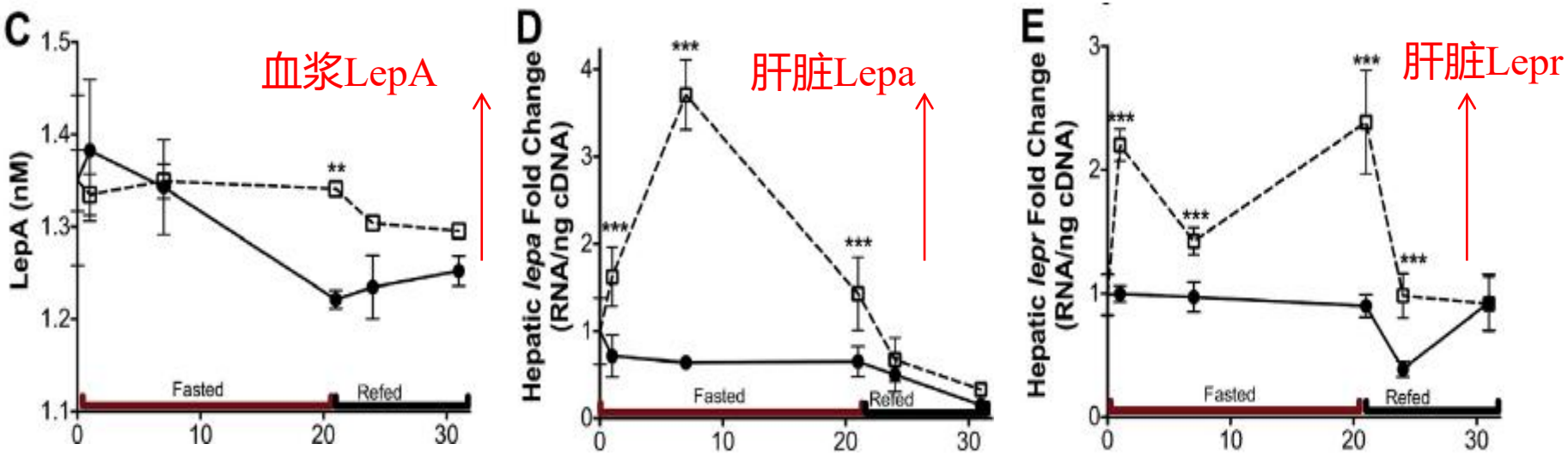
Gene	Accession Number	Forward (5′–3′)	Reverse (5′–3′)	Amplicon Size	Primer Efficiency (%)
Leptin a (*lepa*)	KC354702	GGGTCTCCCAGATCAAGTACGA	TGCCGCCACAGATGAATG	61	99.9
Leptin receptor (*lepr*)	KC354703	AAATTCACCGGAAGCAAACCT	TGCAGCCGGGACTGTGT	56	102.6
Growth hormone (GH)	M26916	GCAACGTCAGCTCAACAAAA	CAGCCTTGGTGAAATCTGGT	202	100.3
Insulin-like growth factor-1 (IGF-1)	EU272149	CCAAGAGCACCCAAGGTTAG	TTTGTCCATTTGCTCCTTCC	206	105.3
Insulin-like growth factor-1 (IGF-2)	ABY88873	GAACGCAGAACAGCAGAATG	GTTGTTACCCCTGCTGGTTG	200	105.4
GH receptor 1 (GHR1)	AY973232	TAAGAAAGAGCCTCCTACCA	ACTGTCGCTGAATGTCCAAT	377	101.3
GH receptor 2 (GHR2)	AY973233	AGCACTGAGACGCCAAACGA	CCAAAGATGAGCAAAGCCAC	494	102.7
Ribosomal 18 s	U67340	ATTACTGGCTACACCGAGCG	AGTCCTGACTCTCCTCCGTC	252	92

Leptin对禁食和复喂的响应



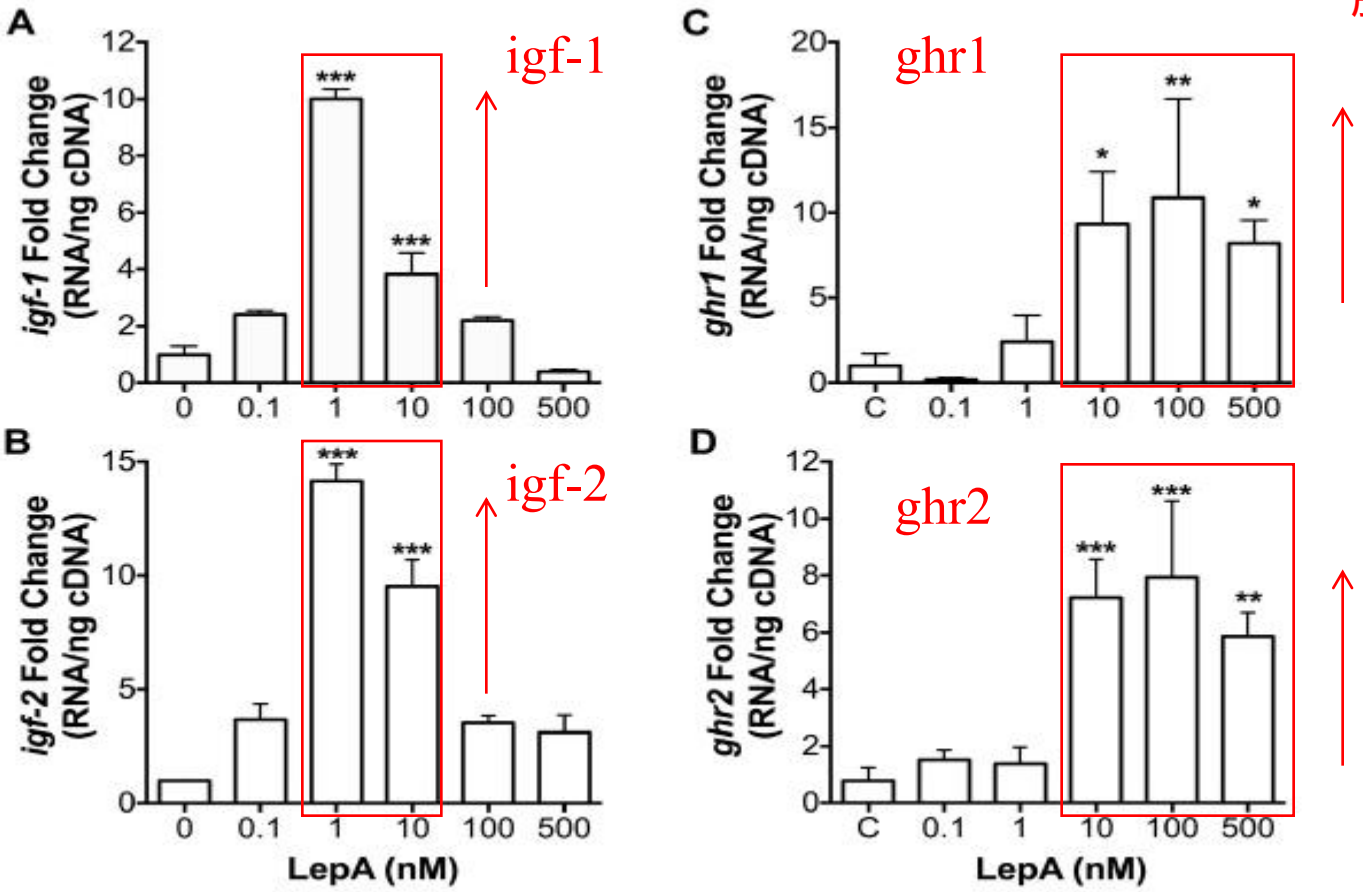
体重

肝体比



禁食期间，血浆lepA水平和肝脏lepa及其受体表达量显著增加，复喂后，恢复到正常水平，表明代谢状态的改变增强了leptin的敏感性,leptin是一种关键的代谢分解因子。

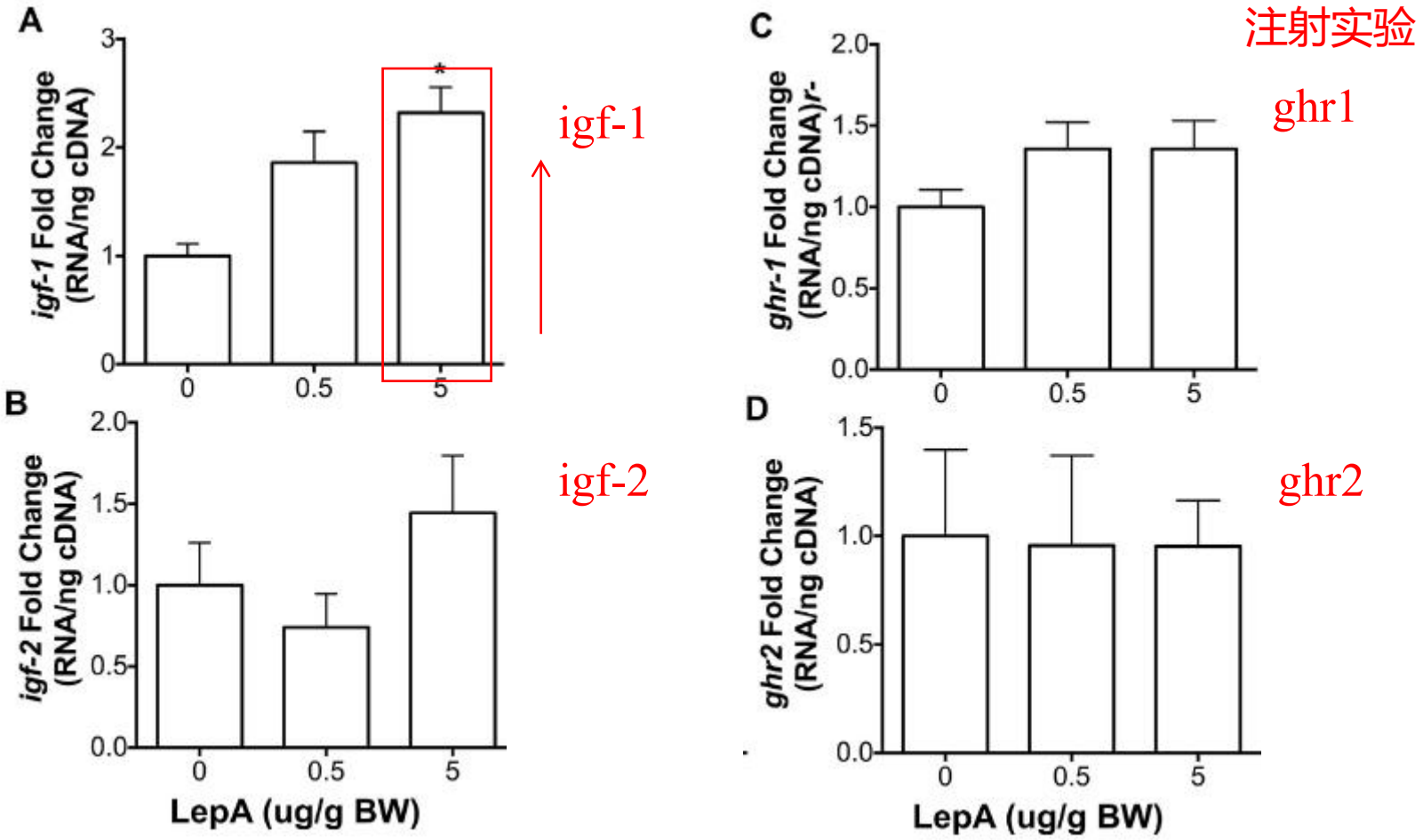
rtLepA在体外对肝脏IGFs和GHRs表达的调控作用



在体外，leptin可显著促进IGFs和GHRs的表达，说明leptin对IGF和GHR有调控作用。

rtLepA在体内对肝脏IGFs和GHRs表达的调控作用

肝脏



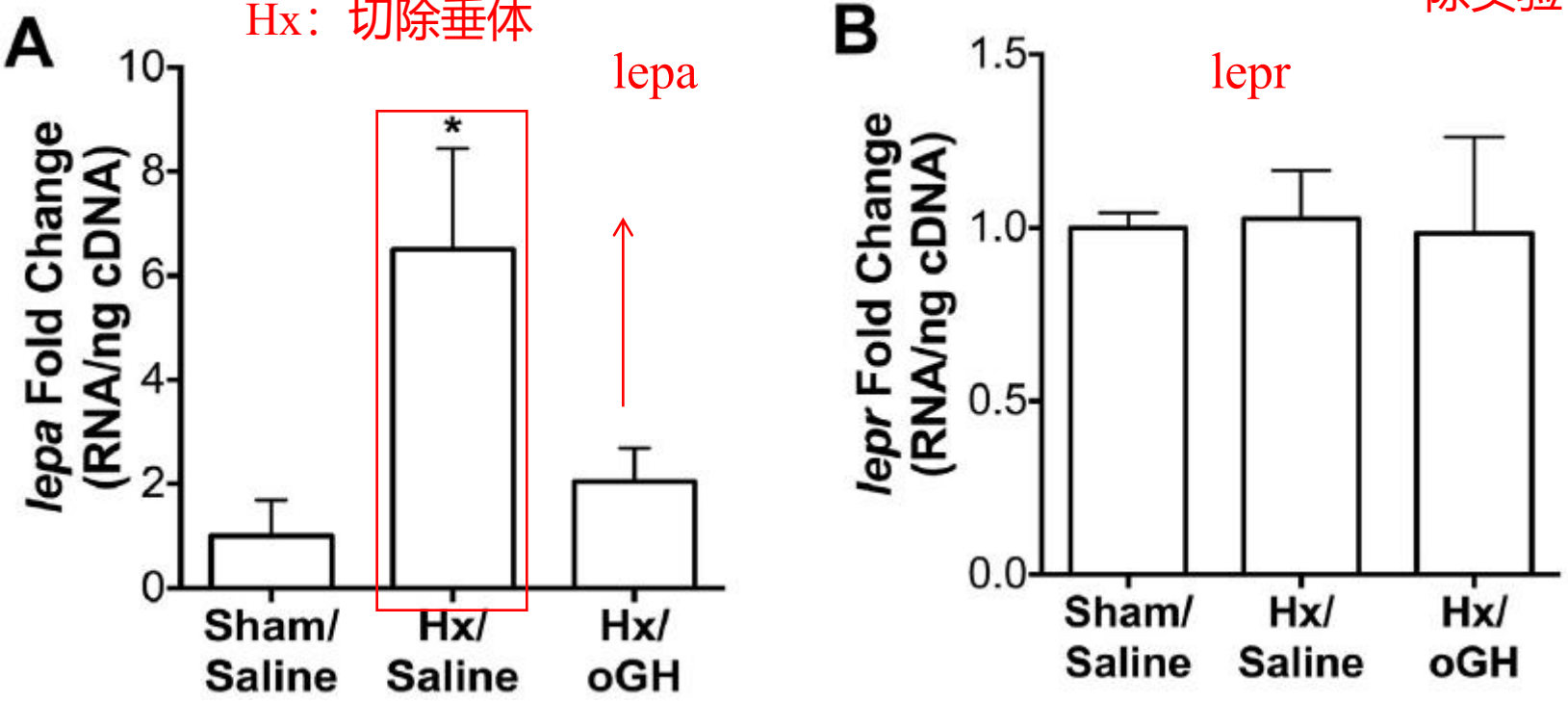
在体内，外源leptin仅显著促进了igf-1的表达，而对igf-2，ghr1和ghr2无影响，这说明内源性的一些因子可能抑制了ghr1和ghr2的转录和翻译。

GH在体内对肝脏lepa和lepr表达的调控作用

肝脏

Sham：不切除垂体
Hx：切除垂体

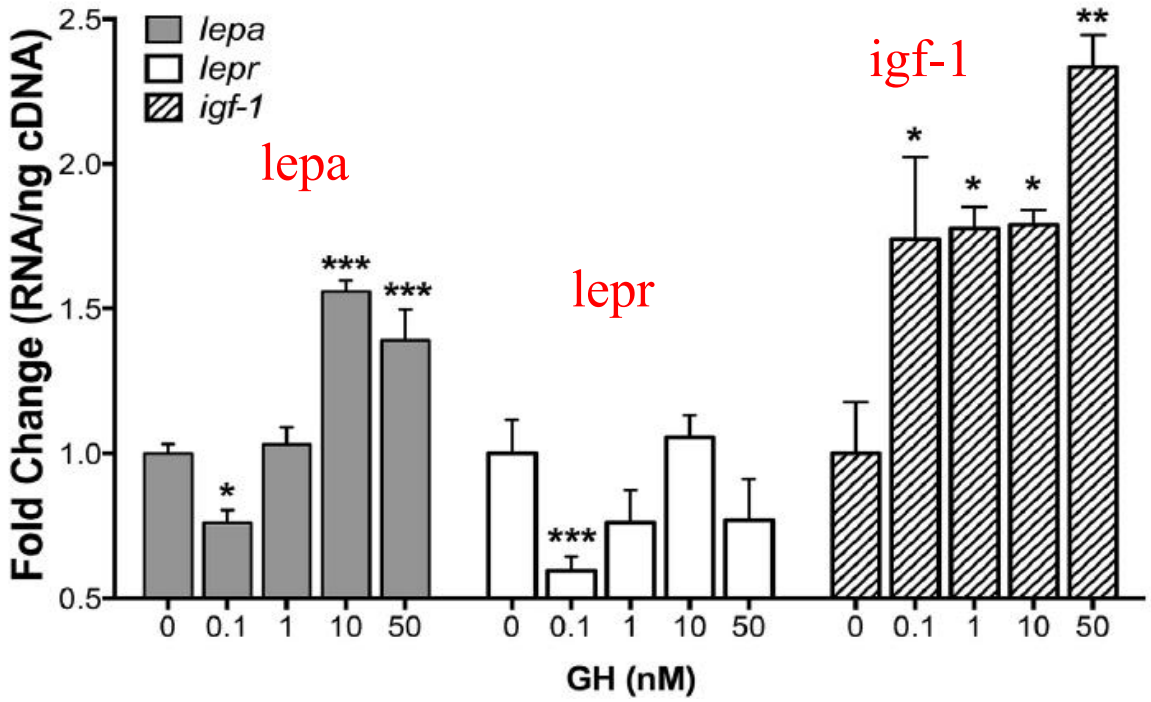
垂体切除实验



垂体切除后，与全鱼相比，leptin表达量显著升高，而垂体切除注射oGH后，leptin表达量恢复到全鱼的水平，说明GH可抑制肝脏leptin的表达。

GH在体外对肝脏lepa和lepr表达的调控作用

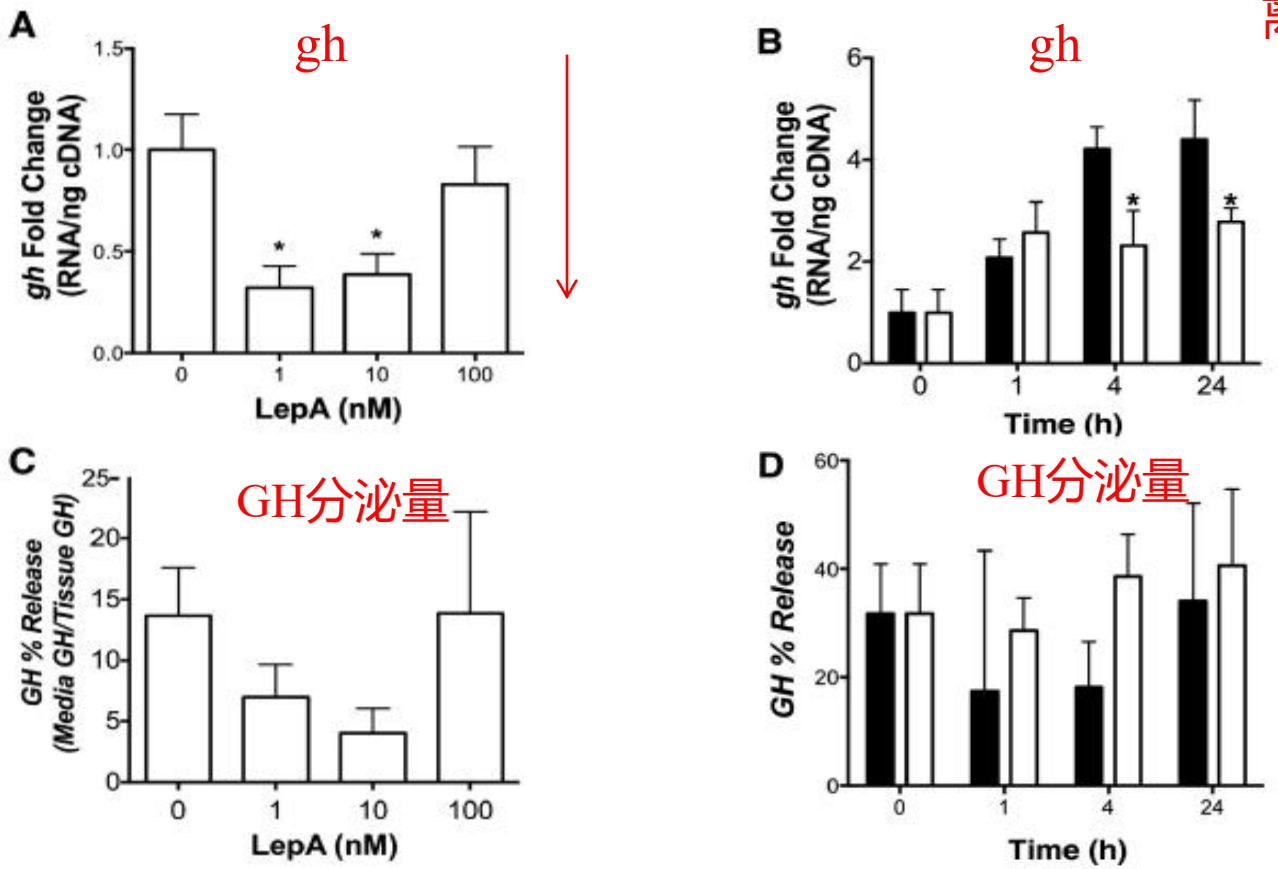
肝细胞离体实验



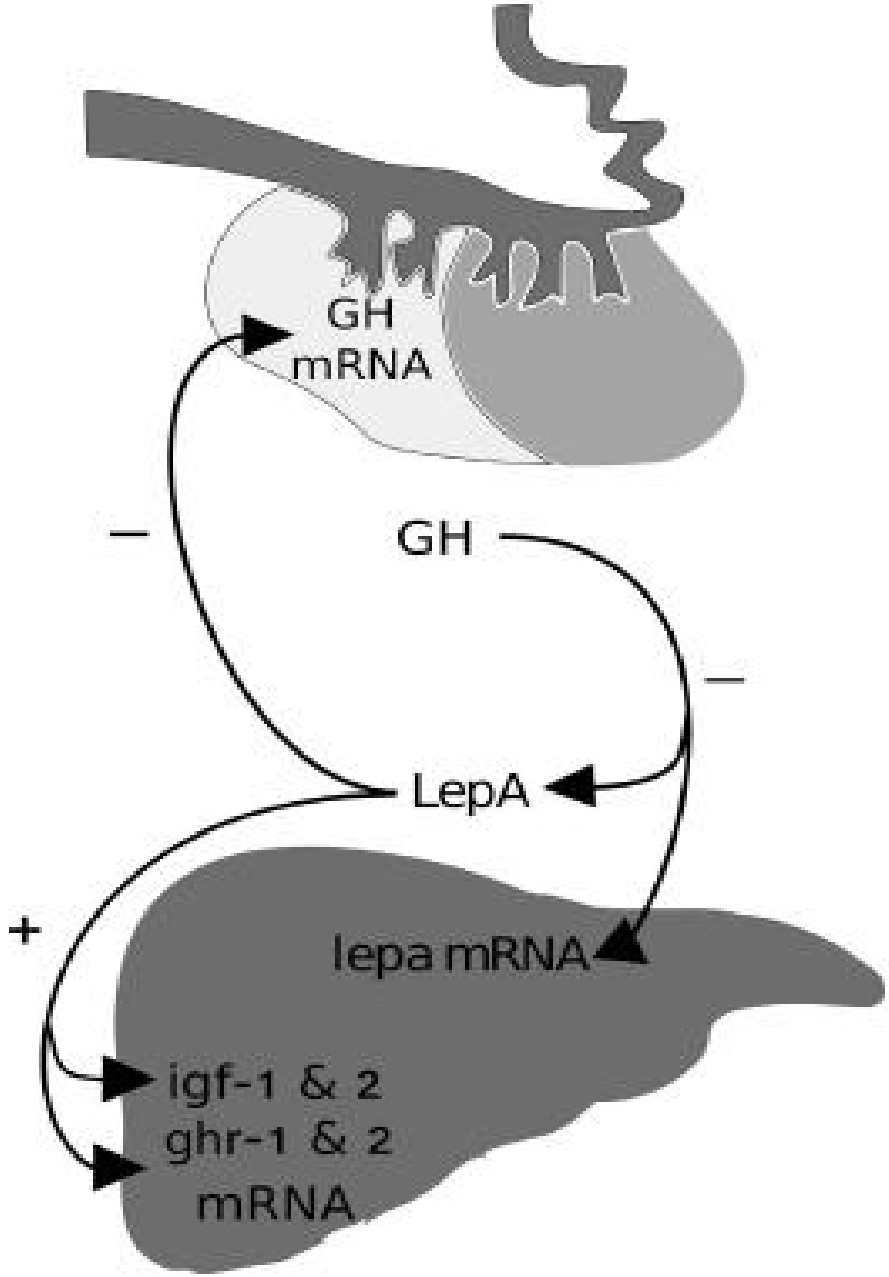
GH在低浓度抑制lepa及其lepr的表达，而高浓度促进leptin的表达，GH在所有浓度下均促进igf-1的表达。

Leptin对垂体GHmRNA的表达和GH分泌的调控作用

垂体外侧部
离体实验



Leptin抑制GH的表达，对其分泌无影响,这可能是leptin抑制了GH的转录及合成所致。



在生理水平上，GH降低肝脏lepa mRNA的表达和lepA的分泌，而在禁食期，lepA降低垂体GH mRNA的表达,促进IGF和GHR mRNA的表达，使其保持在阈值水平，以便复喂后，肝体生长轴快速启动，促进生长。

04

结论

Conclusion

01

leptin的合成和分泌及其受体的表达对代谢状态是敏感的，其表达量随禁食和饲喂时间的增加而增加.

02

leptin可能在肝脏局部发挥作用，刺激肝脏GHR和IGFs基因的表达，进而限制这些因子的代谢分解.

03

GH抑制leptin的表达，leptin减弱GH转录物积累，这表明GH和leptin可能相互作用以调节鱼的能量稳态和代谢.

05

思考

Thinking

1.本研究使我对生长轴关键基因之间的相互作用有了更深的了解。

2.本研究为其他鱼类生长和代谢调控机制的研究提供了参考。


THANK