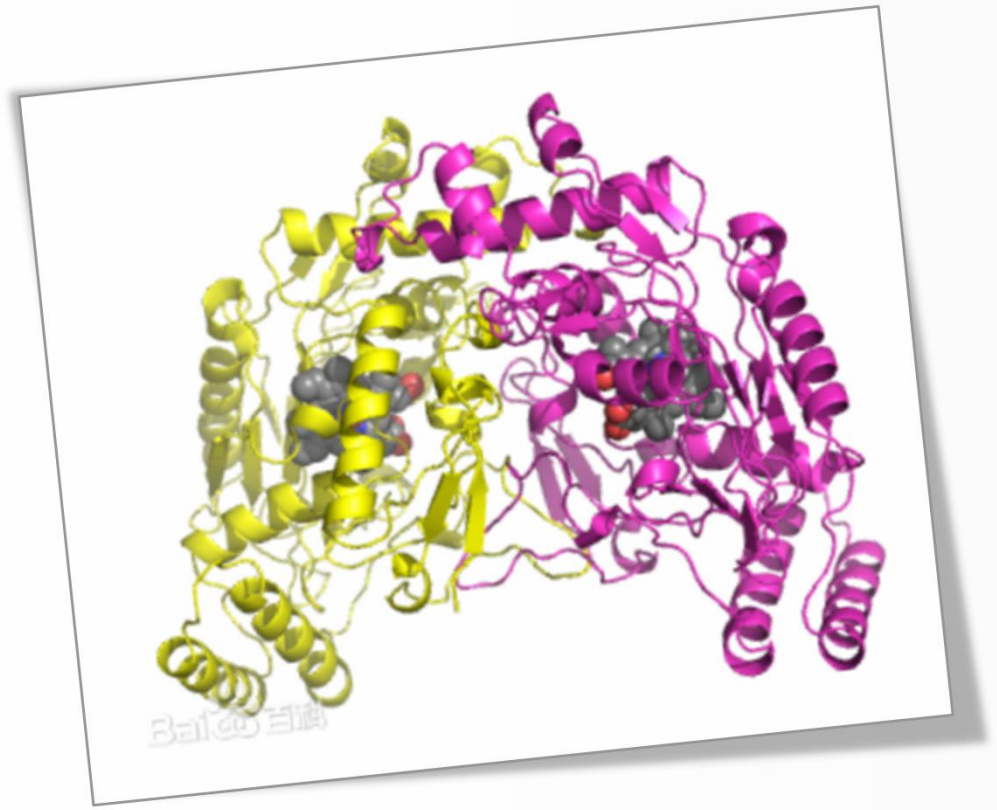
河南师范大学

读书报告

闫潇

2017.5.14



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ORIGINAL RESEARCH COMMUNICATION

Central Apelin Controls Glucose Homeostasis *via* a Nitric Oxide-Dependent Pathway in Mice

抗氧化剂与氧化还原信号 IF:7.093

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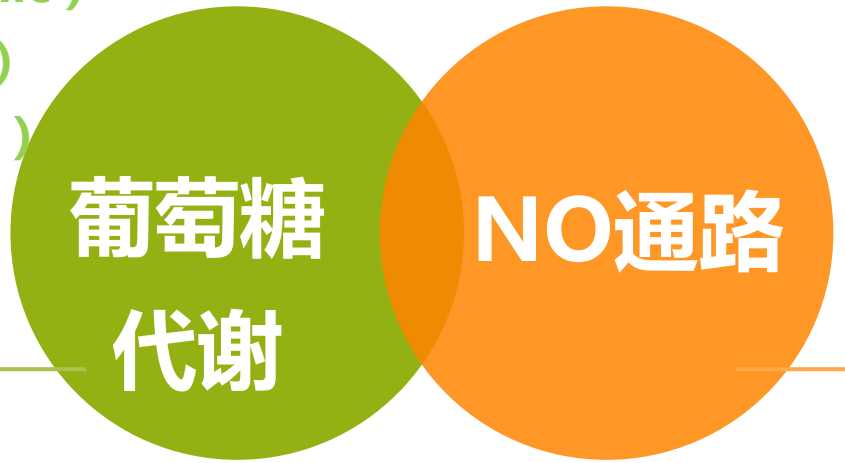
Abstract

Aims: Apelin and its receptor have emerged as promising targets for the treatment of insulin resistance. Indeed, peripheral administration of apelin stimulates glucose utilization and insulin sensitivity via a nitric oxide (NO) pathway. In addition to being expressed on peripheral metabolically active adipose tissues, apelin is also found in the brain. However, no data are available on the role of central effects of apelin on metabolic control. We studied glucose metabolism in response to acute and chronic intracerebroventricular (i.c.v.) injection of apelin performed in normal and obese/diabetic mice. ***Results:*** We demonstrate that i.c.v. injection of apelin into fed mice improves glucose control via NO-dependent mechanisms. These results have been strengthened by transgenic (eNOS-KO mice), pharmacological (L-NMMA i.c.v. treated mice), and real-time measurement of NO release with amperometric probes detection. High-fat diet-fed mice displayed a severely blunted response to i.c.v. apelin associated with a lack of NO response by the hypothalamus. Moreover, central administration of high dose apelin in fasted normal mice provoked hyperinsulinemia, hyperglycemia, glucose intolerance, and insulin resistance. ***Conclusion:*** These data provide compelling evidence that central apelin participates in the regulation of glucose homeostasis and suggest a novel pathophysiological mechanism involved in the transition from normal to diabetic state. *Antioxid. Redox Signal.* 15, 1477–1496.

注射过程（急性acute VS 慢性chronic）
注射剂量（低浓度LD VS 高浓度HD）
营养状态（禁食Fasted VS 喂食Fed）
生理状态（生理NC VS 病理HFD）
昼夜节律



eNOS KO
eNOS KO SNAP
L-NMMA

中枢Apelin通过NO-依赖途径控制小鼠葡萄糖稳态

正常 → apelin → 糖尿病

实验方法

- 急性注射（Acute injections）：apelin；NOS inhibitor：L-NMMA
- 慢性灌注（Chronic perfusion of apelin）
- 实时电流表测定NO（Real-time amperometric NO measurements）
- 口服葡萄糖耐量试验（Oral glucose tolerance test）
- 胰岛素耐受试验（ITT）
- 正常血糖高胰岛素 钳夹（Euglycemic hyperinsulinemic clamp）
- ELISA（试剂盒）：胰岛素；血浆apelin
- 实时定量测定下丘脑c-Fos表达变化（Quantification of c-Fos expression in the hypothalamus）
- Western blot analysis：Phospho eNOS / Total eNOS

一些激素只在高血糖状态下发挥作用，在正常状态下不发挥作用，如GLP-1。

假设：

Apelin能够发挥作用取决于营养状态。

VS

1 Dose-response effect on glycemia in response to i.c.v. apelin in fasted or fed mice

禁食

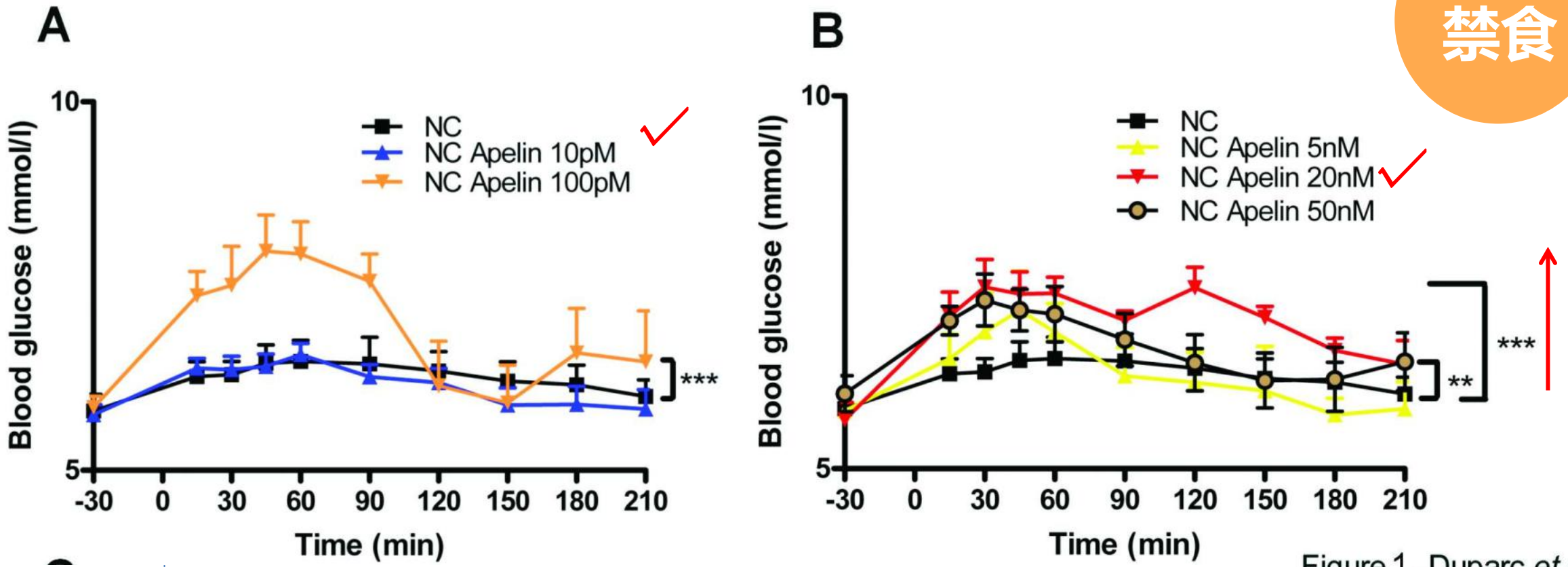
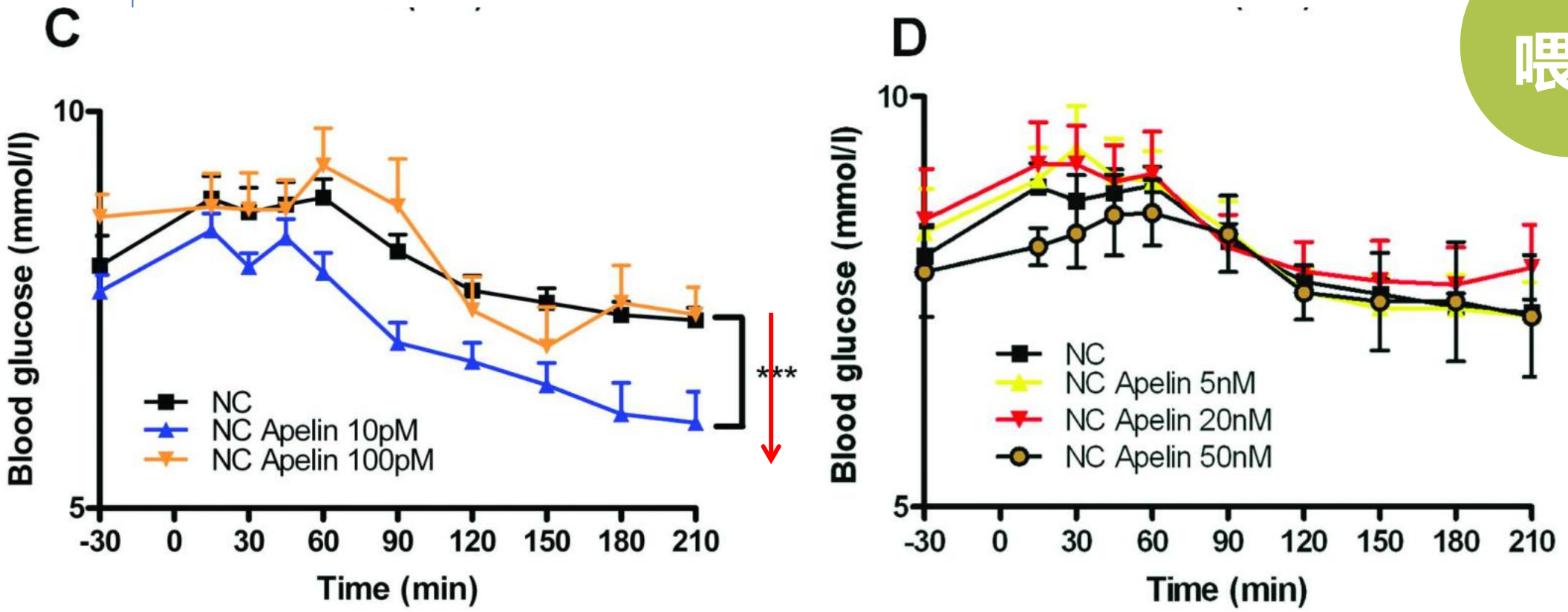


Figure 1, Duparc *et al.*

禁食状态下，LD（10pM）apelin对血糖无影响，HD（20nM） apelin 升高血糖。

1 Dose-response effect on glycemia in response to i.c.v. apelin in fasted or fed mice

喂食



喂食状态下，LD apelin对降低血糖，HD apelin 不影响血糖。

Figure 1 , Duparc *et al.*

1

mice

禁食

喂食

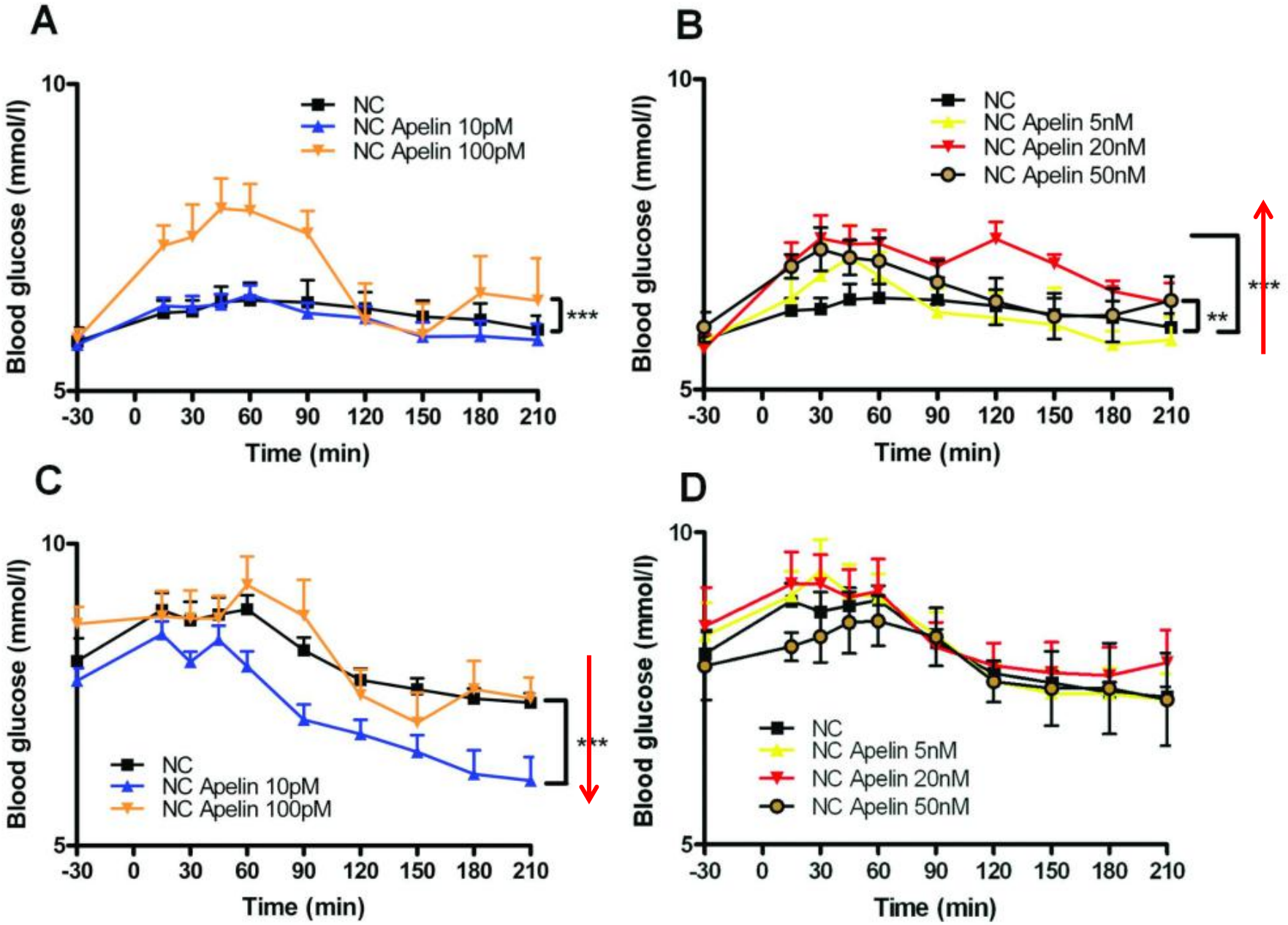


Figure 1 , Duparc *et al.*

2 Effect of acute i.c.v. LD apelin injection on peripheral glucose metabolism in physiological and pathophysiological conditions

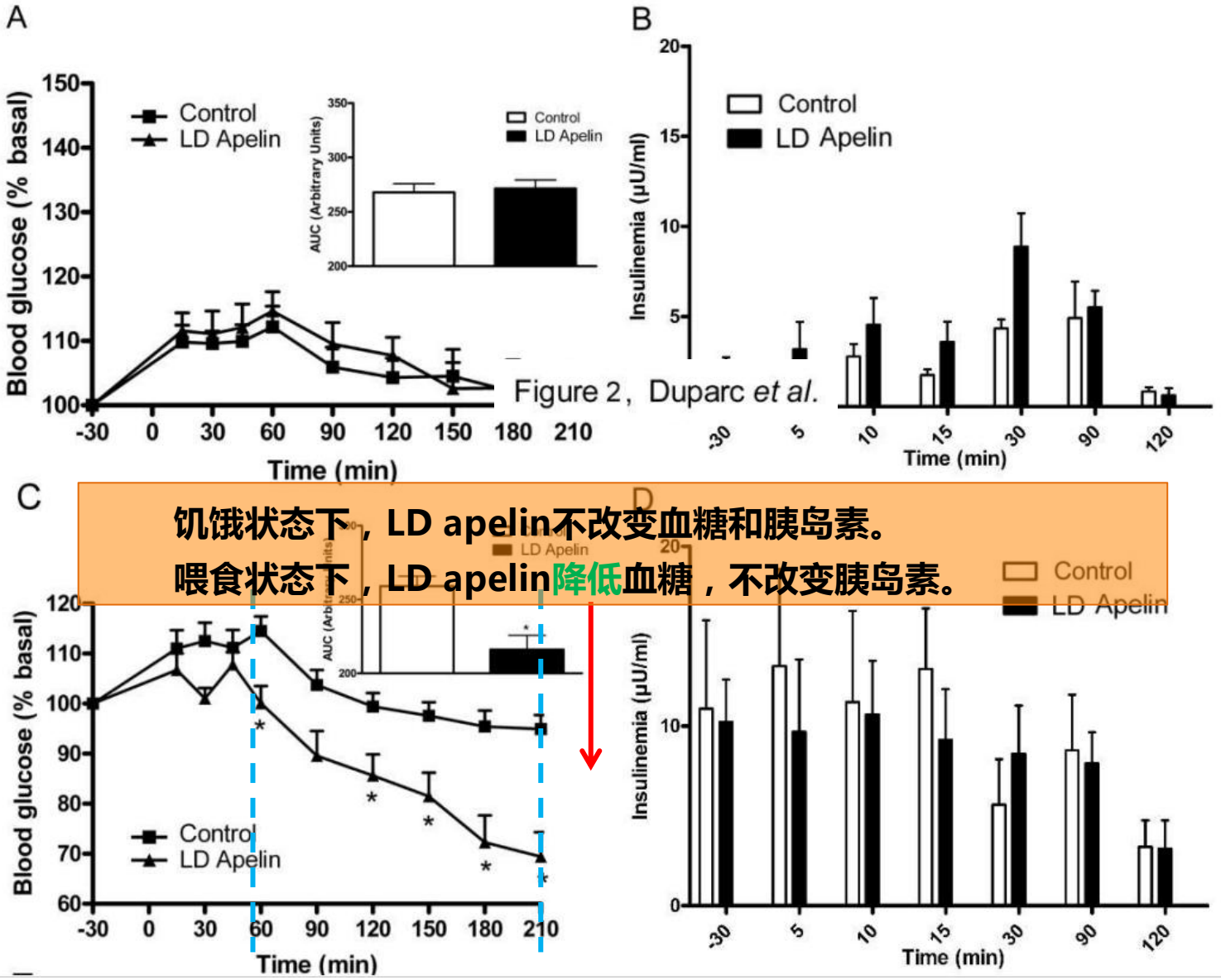


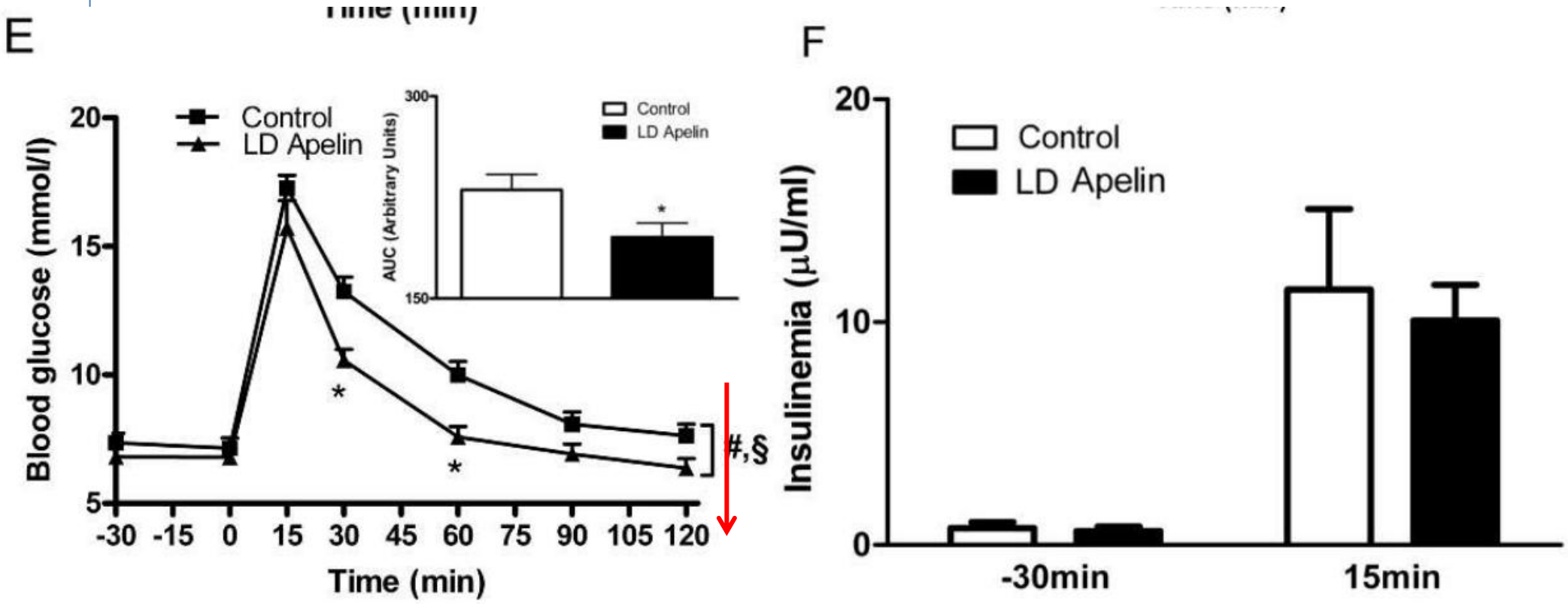
Figure 2，Duparc *et al.*

禁食

喂食

Figure 2，Duparc *et al.*

2 Effect of acute i.c.v. LD apelin injection on peripheral glucose metabolism in physiological and pathophysiological conditions



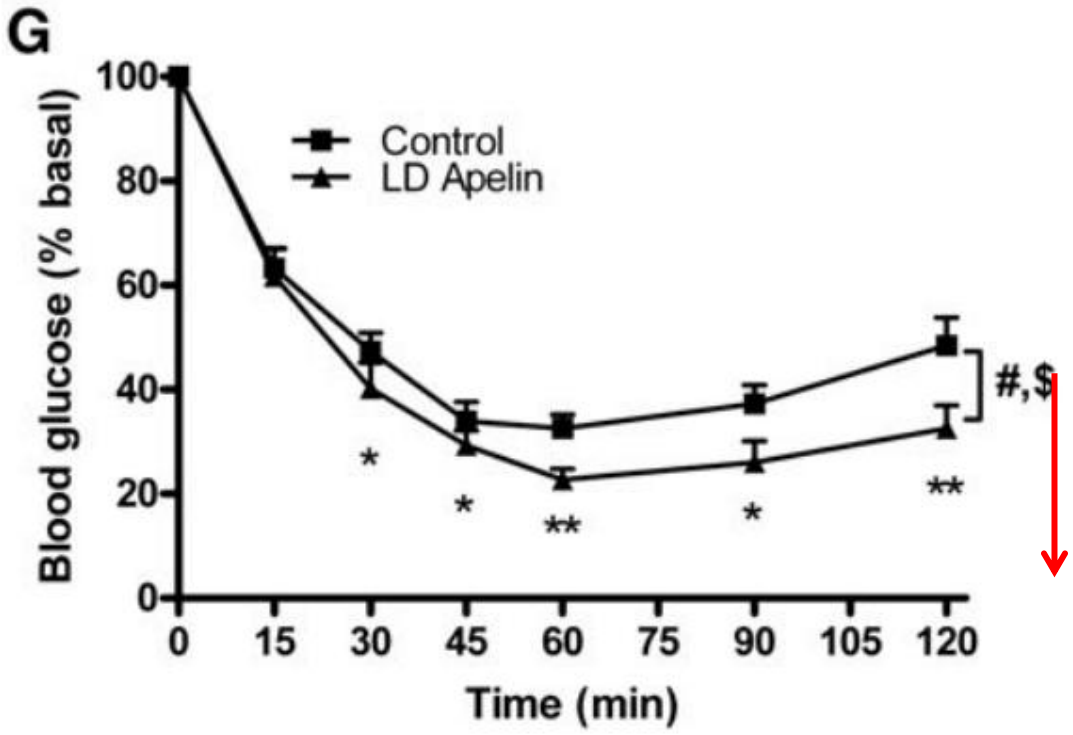
OGTT

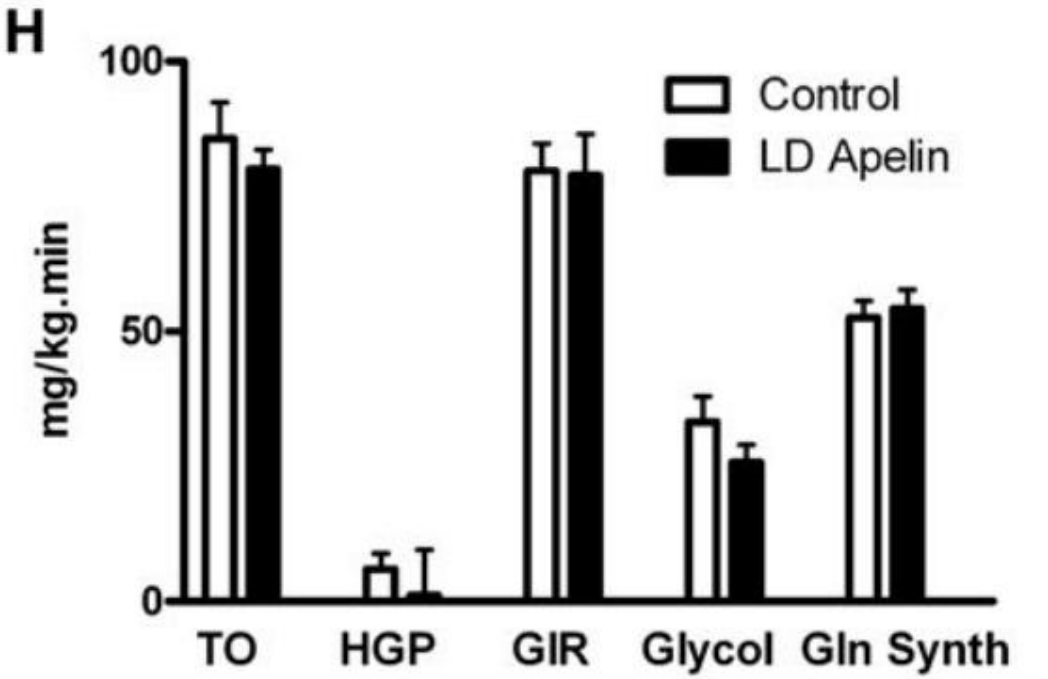
口服葡萄糖耐量试验

Figure 2，Duparc *et al.*

LD apelin显著改善糖耐量，不影响胰岛素。

2 Effect of acute i.c.v. LD apelin injection on peripheral glucose metabolism in physiological and pathophysiological conditions





Glu周转率　肝Glu产生　Glu输注率　糖酵解　糖异生

ITT
胰岛素耐受实验

euglycemic hyperinsulinemic clamp
正常血糖高胰岛素钳夹实验

Figure 2，Duparc *et al.*

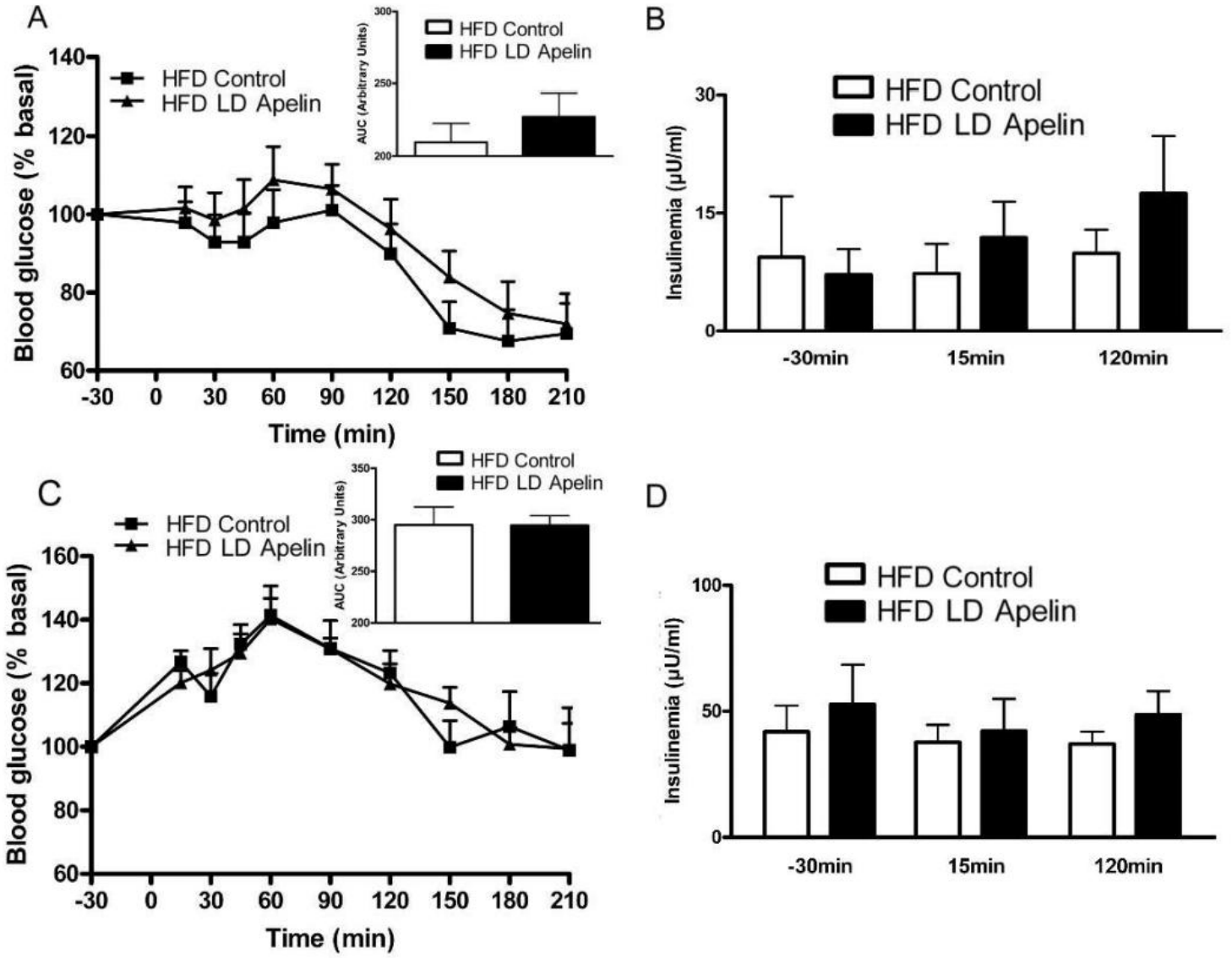
在超生理剂量的胰岛素作用下，LD apelin更加耐受葡萄糖。

在正常生理剂量的胰岛素作用下，LD apelin不改变外周胰岛素敏感性。

在肥胖/糖尿病状态下，常常会发生对代谢中枢调控因子的抵抗，那么肥胖/糖尿病小鼠会不会对apelin出现抵抗呢？

2 Effect of acute i.c.v. LD apelin injection on peripheral glucose metabolism in physiological and pathophysiological conditions

HFD
高糖高脂小鼠



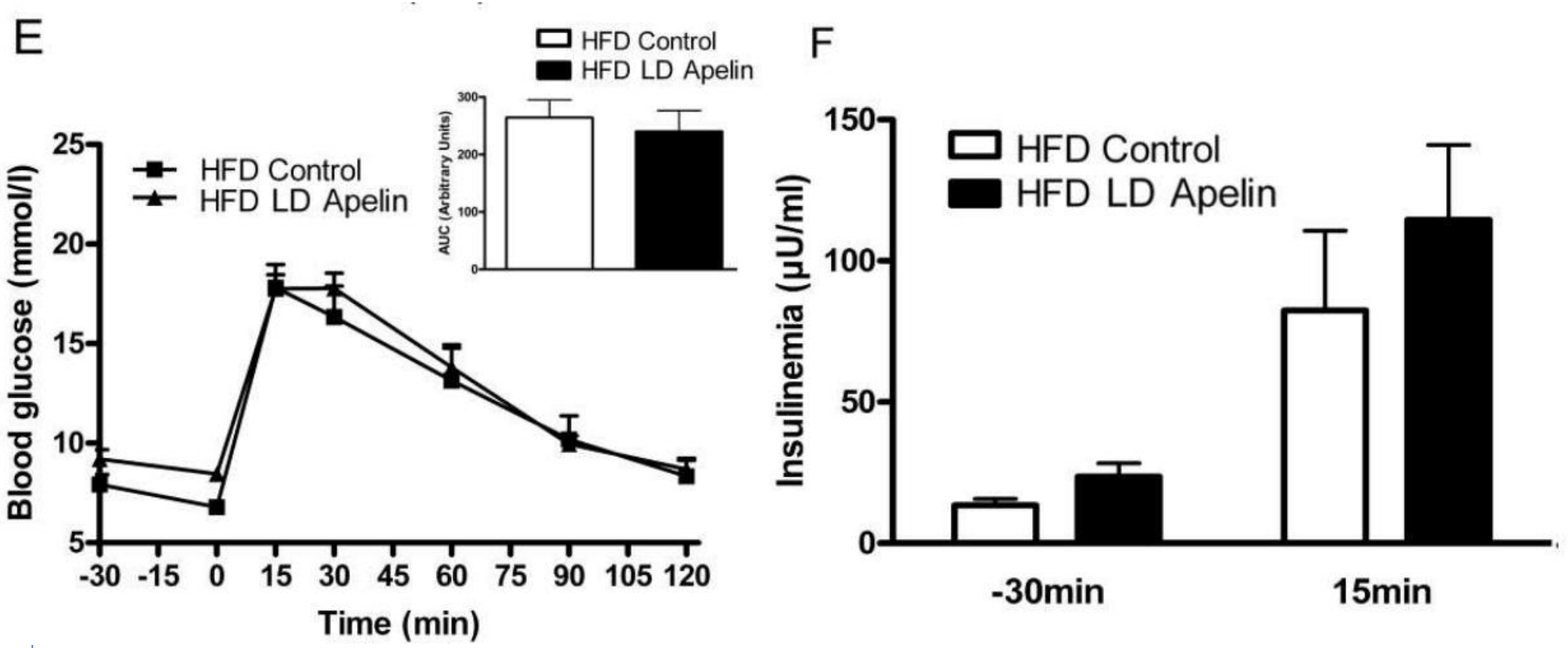
禁食

喂食

Figure 3, Duparc *et al.*

2 Effect of acute i.c.v. LD apelin injection on peripheral glucose metabolism in physiological and pathophysiological conditions

HFD
高糖高脂小鼠

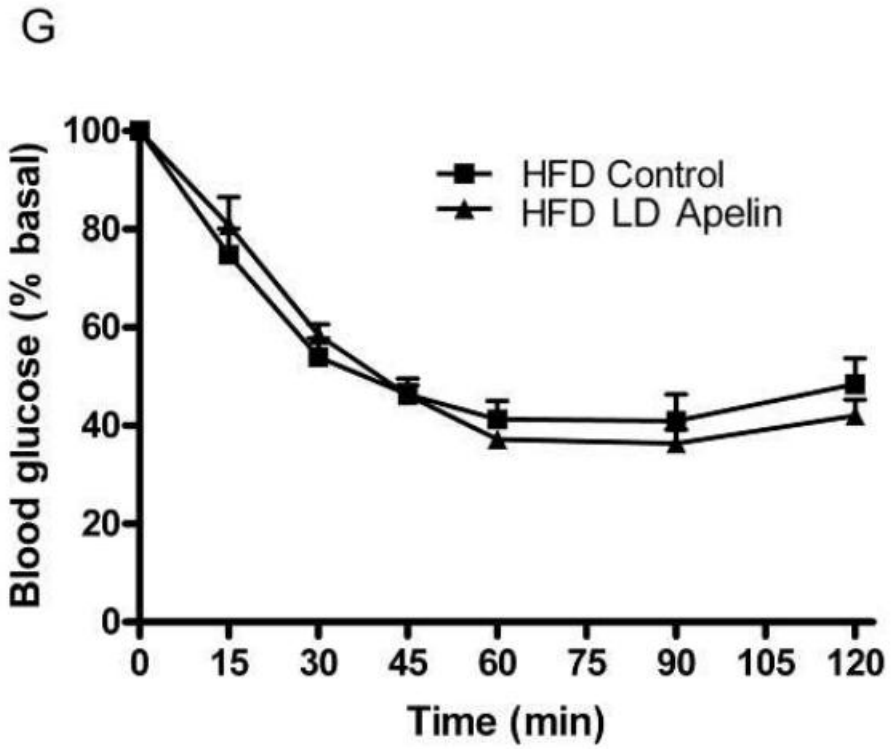


OGTT
口服葡萄糖耐量试验

Figure 3, Duparc *et al.*

2 Effect of acute i.c.v. LD apelin injection on peripheral glucose metabolism in physiological and pathophysiological conditions

HFD
高糖高脂小鼠



ITT
胰岛素耐受实验

LD apelin对葡萄糖稳态的促进作用被HFD条件消除（abolished）了。

Figure 3, Duparc *et al.*

LD apelin不能改善HFD葡萄糖稳态。一个可能的解释是：循环中的apelin对下丘脑产生了不同的刺激。

根据这个假设，我们检测了生理和病理中状态下血浆apelin的昼夜变化。

3 Circadian variations of plasma apelin levels in NC and HFD mice

apelin

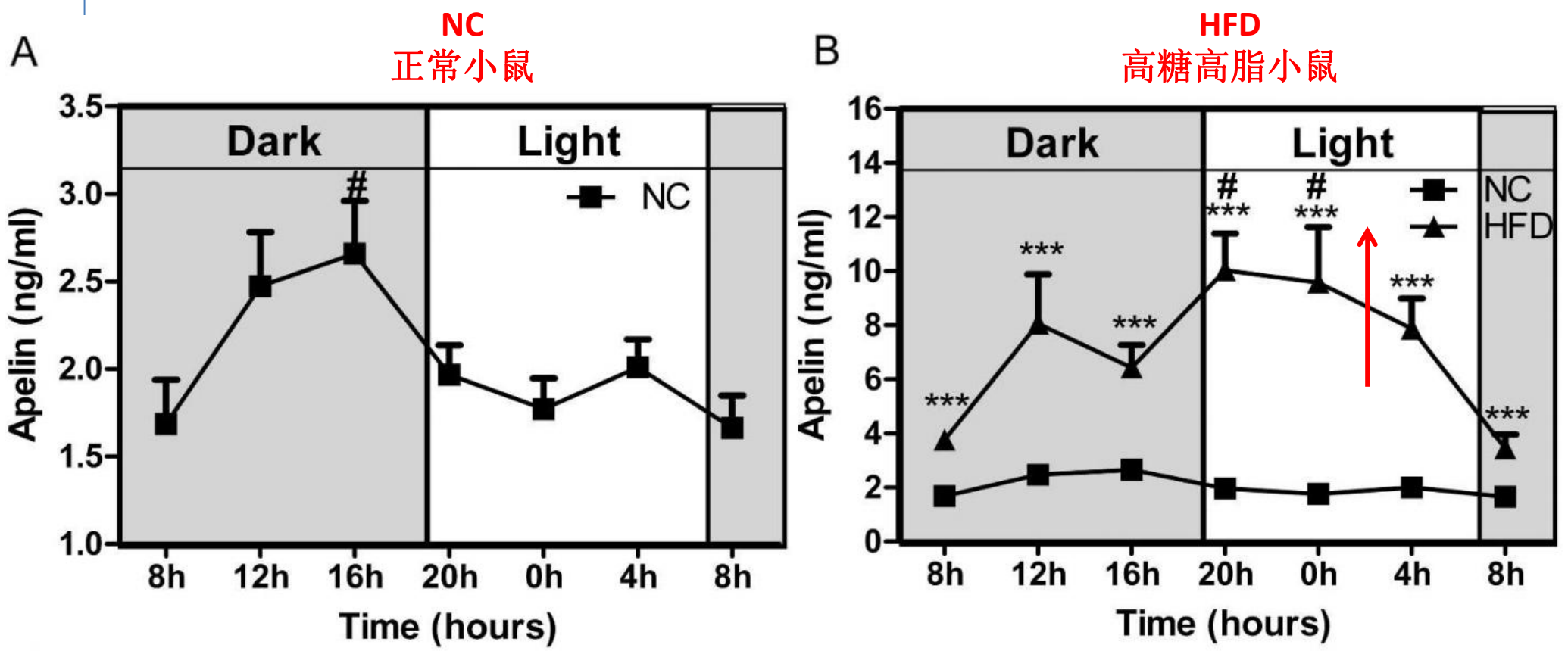


Figure 4, Duparc *et al.*

3 Circadian variations of plasma apelin levels in NC and HFD mice

insulin

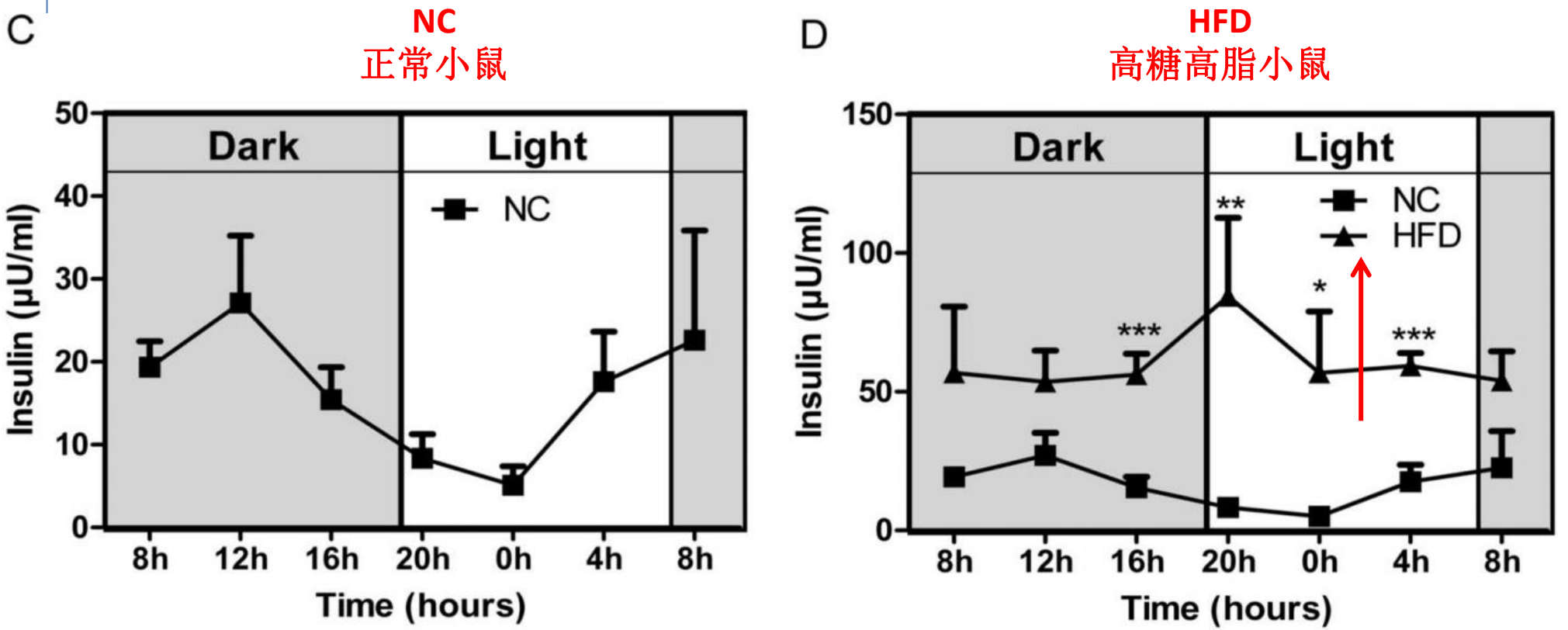


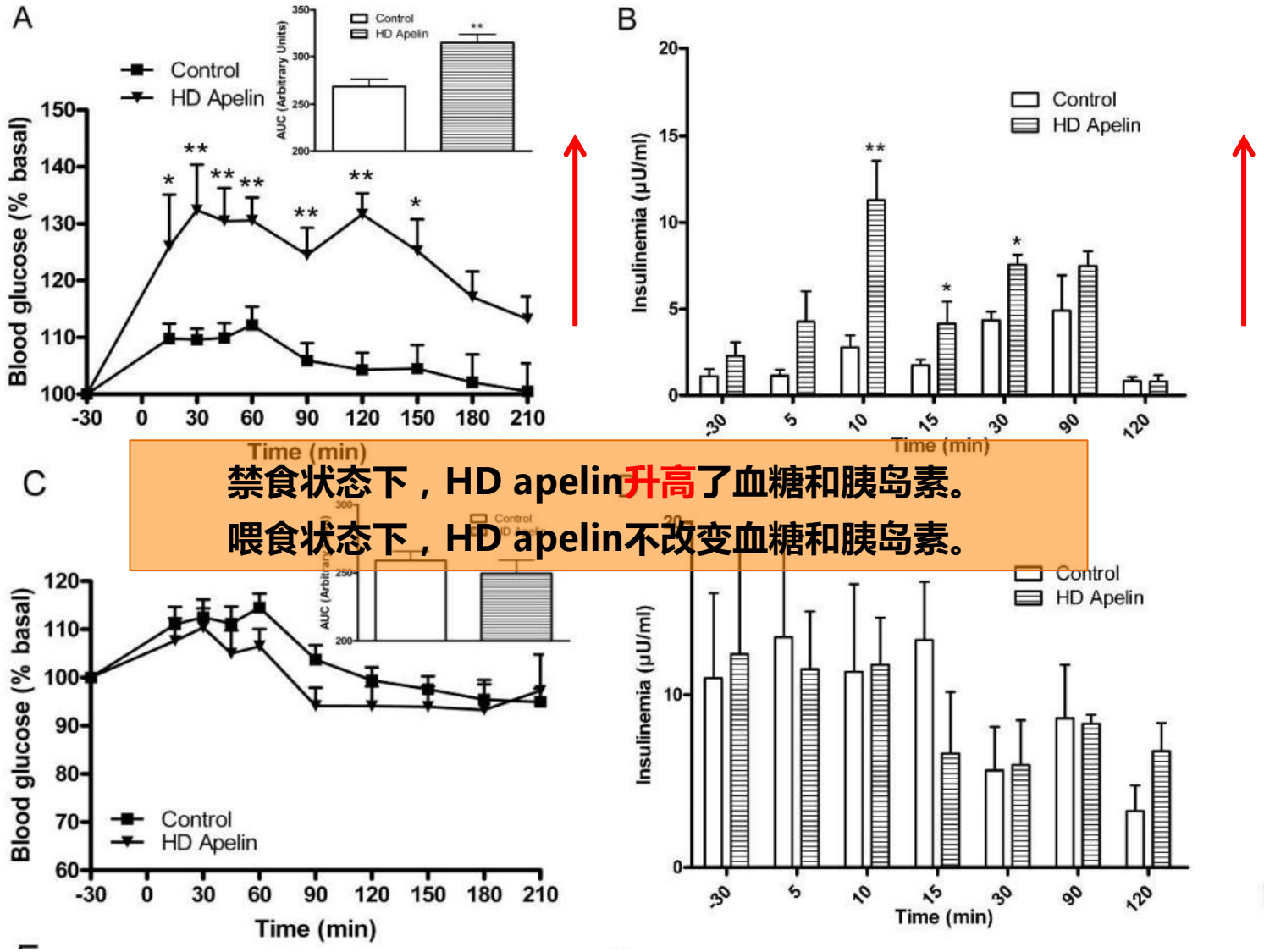
Figure 4, Duparc *et al.*

相对于正常小鼠，HFD小鼠出现昼夜节律的异常，而且血浆apelin和胰岛素都显著升高了！

肥胖/糖尿病状态下，脑神经元发生了改变，而高水平的血浆apelin在其中发挥了重要的作用！

因此，我们检测了在生理和病理条件下，HD apelin对外周葡萄糖代谢的影响。

4 Effect of acute i.c.v. HD apelin injection on peripheral glucose metabolism in physiological and pathophysiological conditions



禁食状态下，HD apelin升高了血糖和胰岛素。

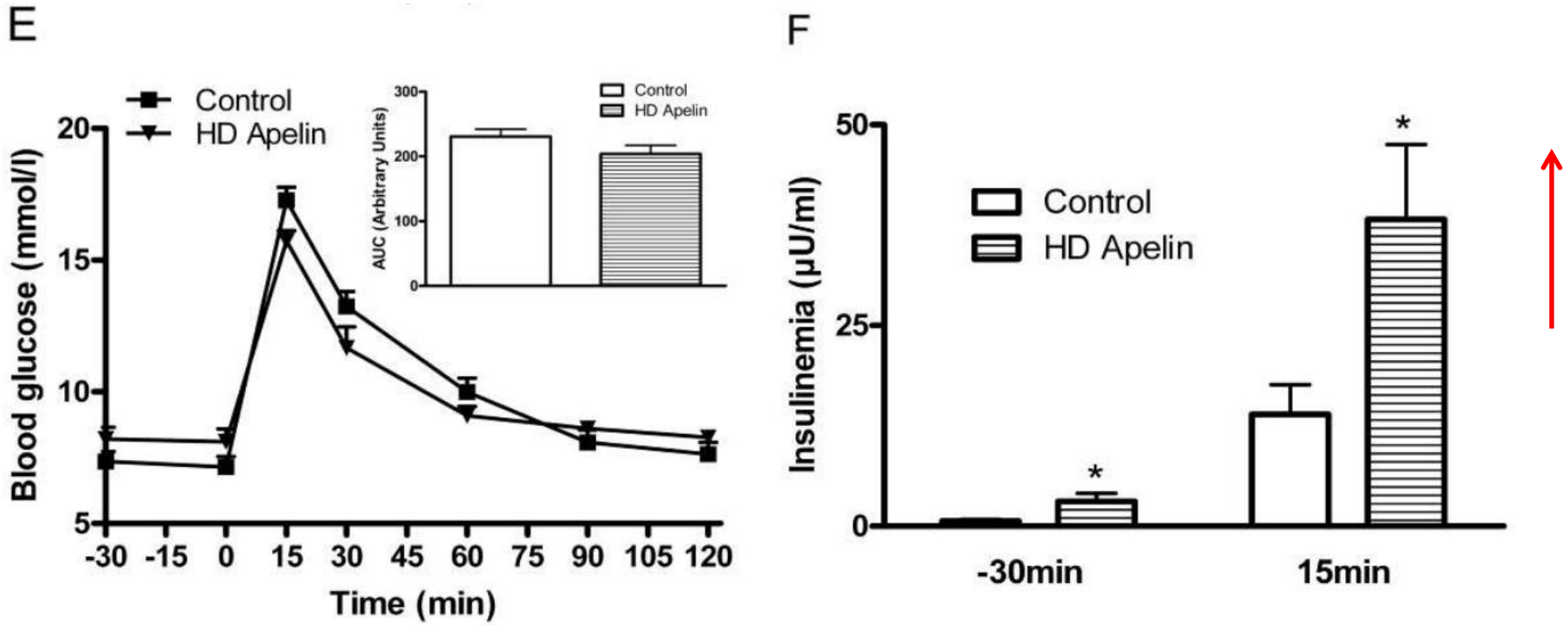
喂食状态下，HD apelin不改变血糖和胰岛素。

禁食

喂食

Figure 5, Duparc *et al.*

4 Effect of acute i.c.v. HD apelin injection on peripheral glucose metabolism in physiological and pathophysiological conditions



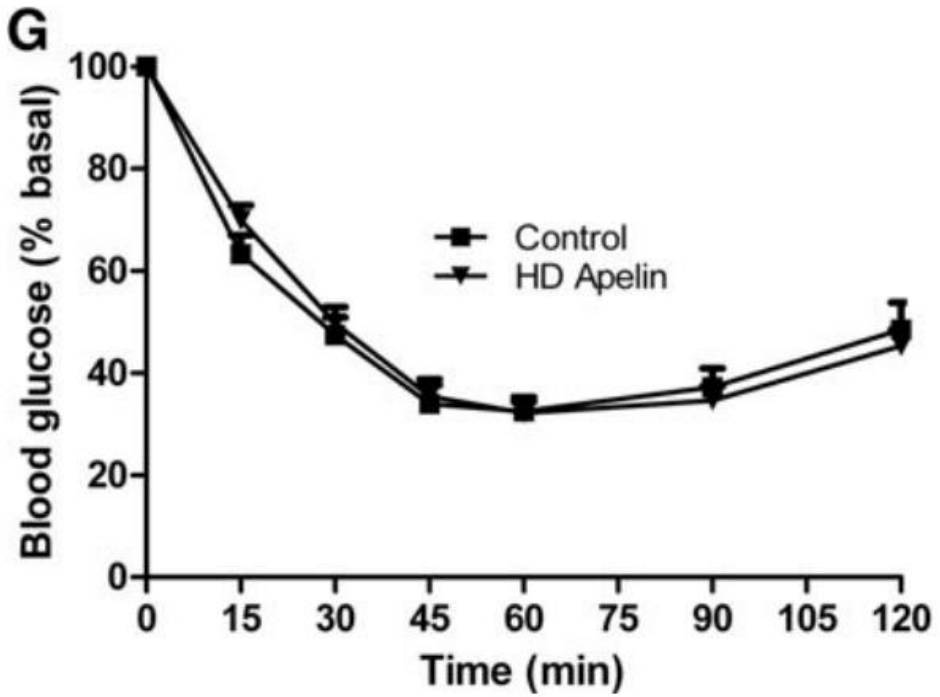
OGTT

口服葡萄糖耐量试验

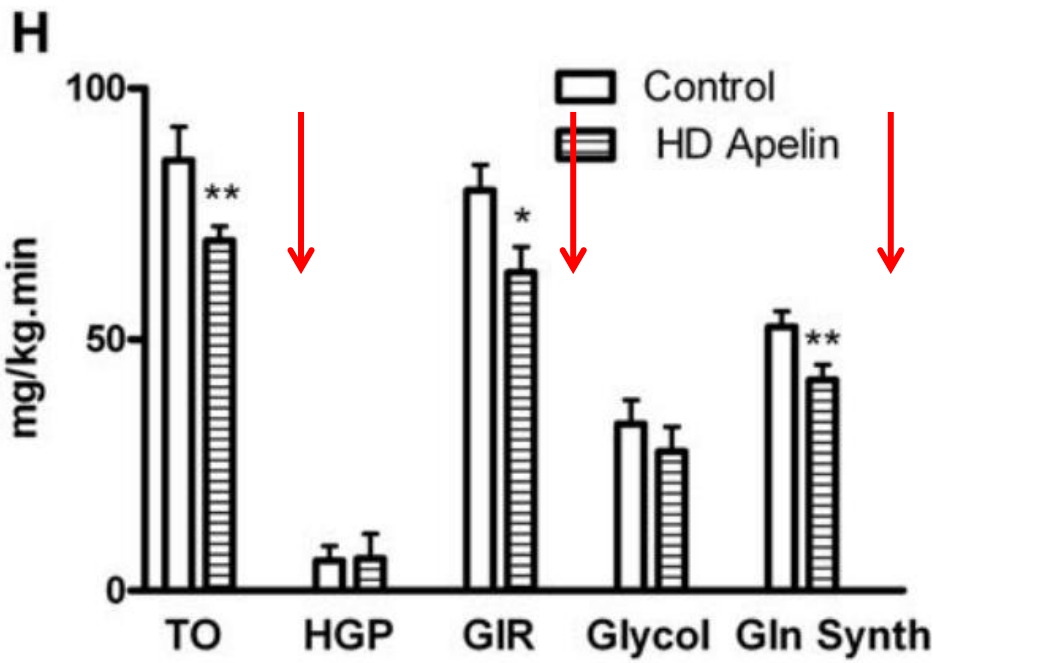
Figure 5, Duparc *et al.*

HD apelin增强了机体胰岛素抵抗。

4 Effect of acute i.c.v. HD apelin injection on peripheral glucose metabolism in physiological and pathophysiological conditions



ITT
胰岛素耐受实验



Glu周转率　肝Glu产生　Glu输注率　糖酵解　糖异生

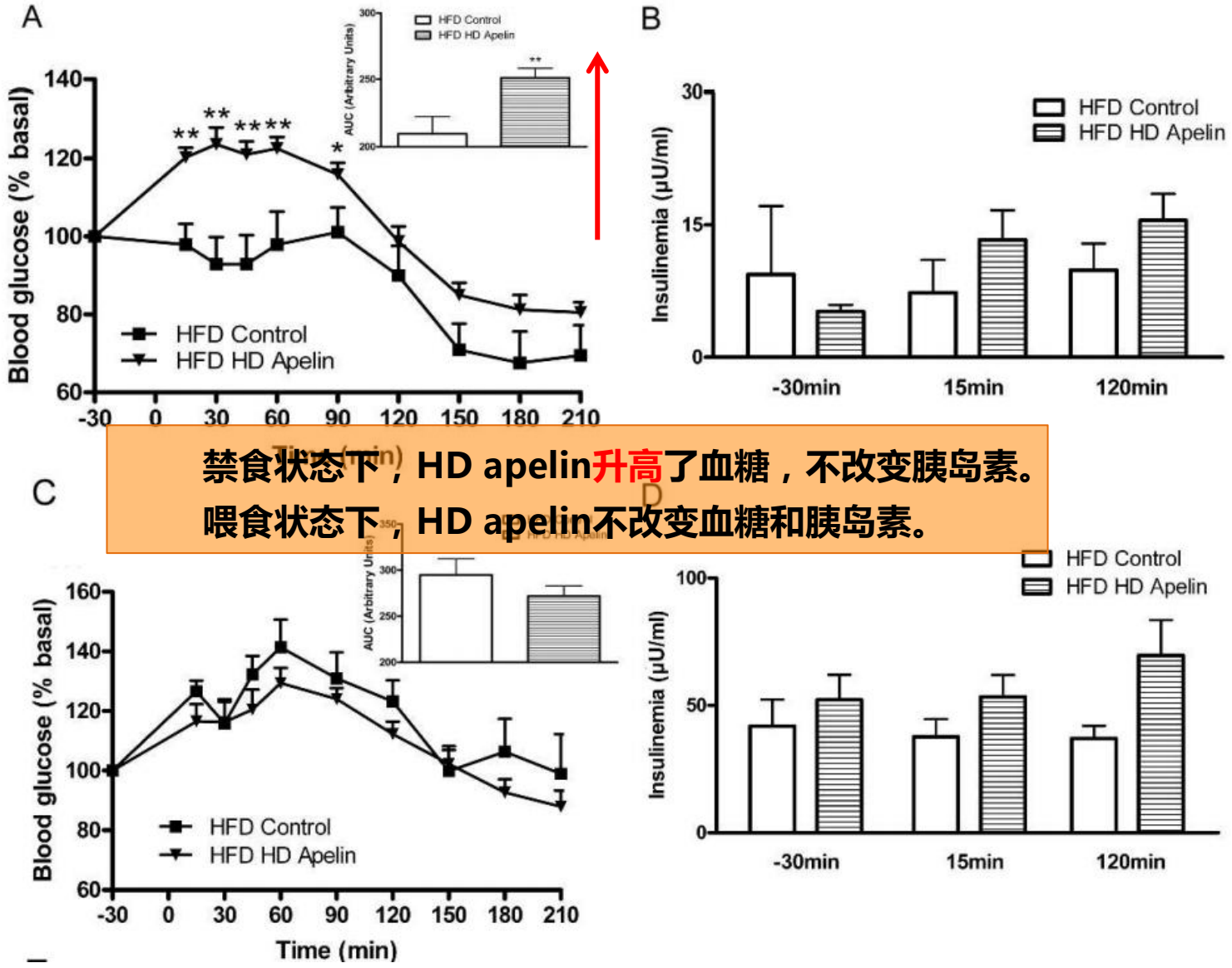
euglycemic hyperinsulinemic clamp
正常血糖高胰岛素钳夹实验

HD apelin降低了机体胰岛素敏感性。

Figure 5, Duparc *et al.*

4 Effect of acute i.c.v. HD apelin injection on peripheral glucose metabolism in physiological and pathophysiological conditions

HFD
高糖高脂小鼠



禁食状态下，HD apelin升高了血糖，不改变胰岛素。
喂食状态下，HD apelin不改变血糖和胰岛素。

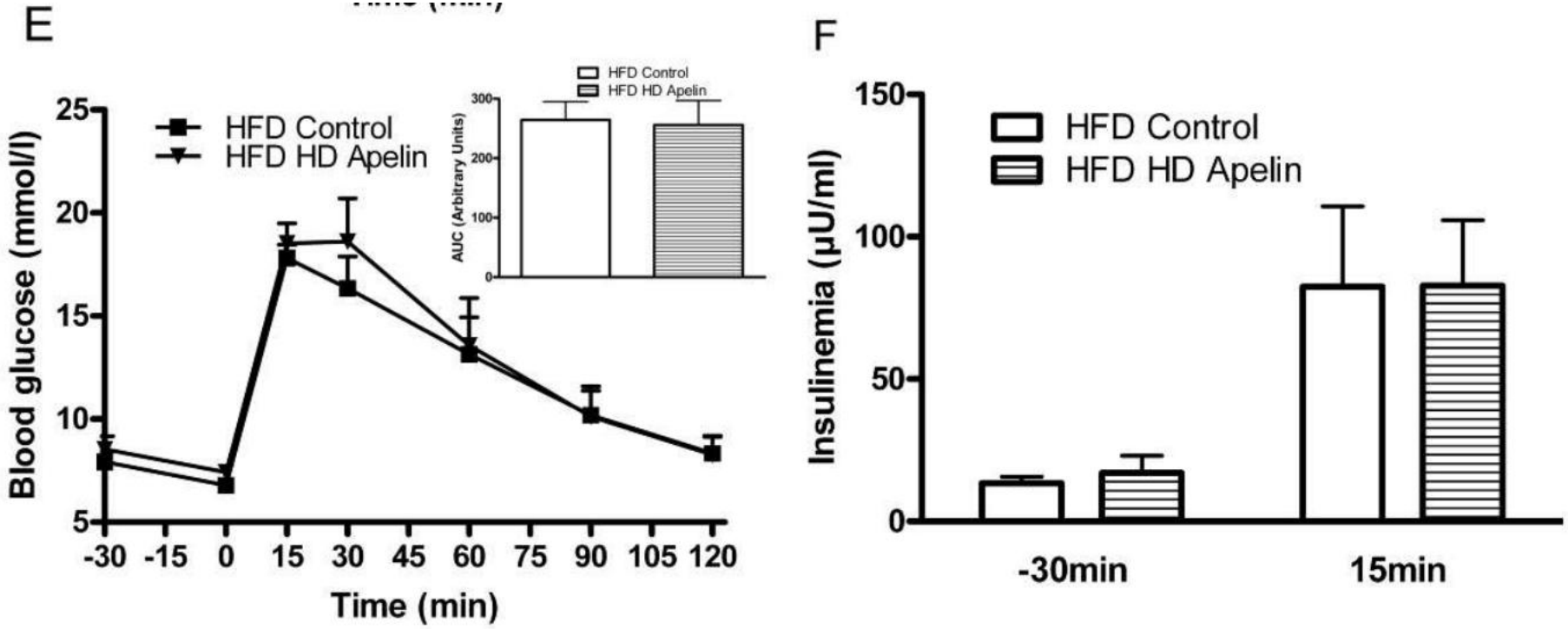
禁食

喂食

Figure 6，Duparc *et al.*

4 Effect of acute i.c.v. HD apelin injection on peripheral glucose metabolism in physiological and pathophysiological conditions

HFD
高糖高脂小鼠

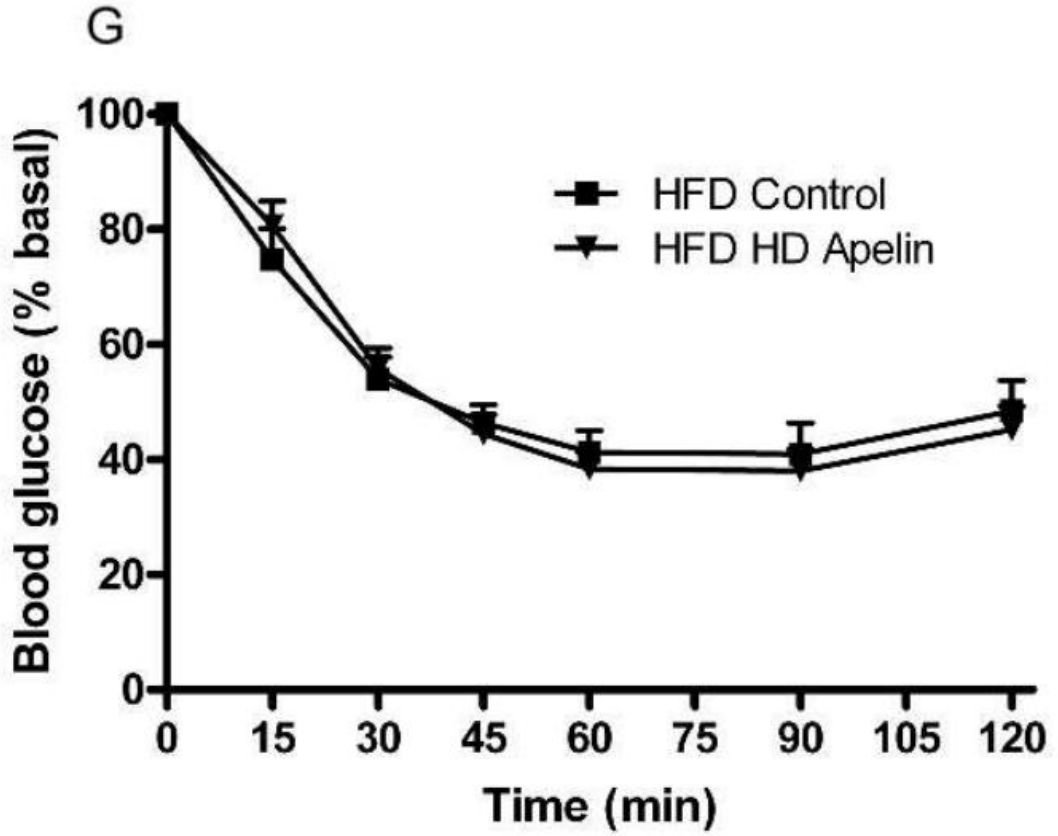


OGTT
口服葡萄糖耐量试验

Figure 6, Duparc *et al.*

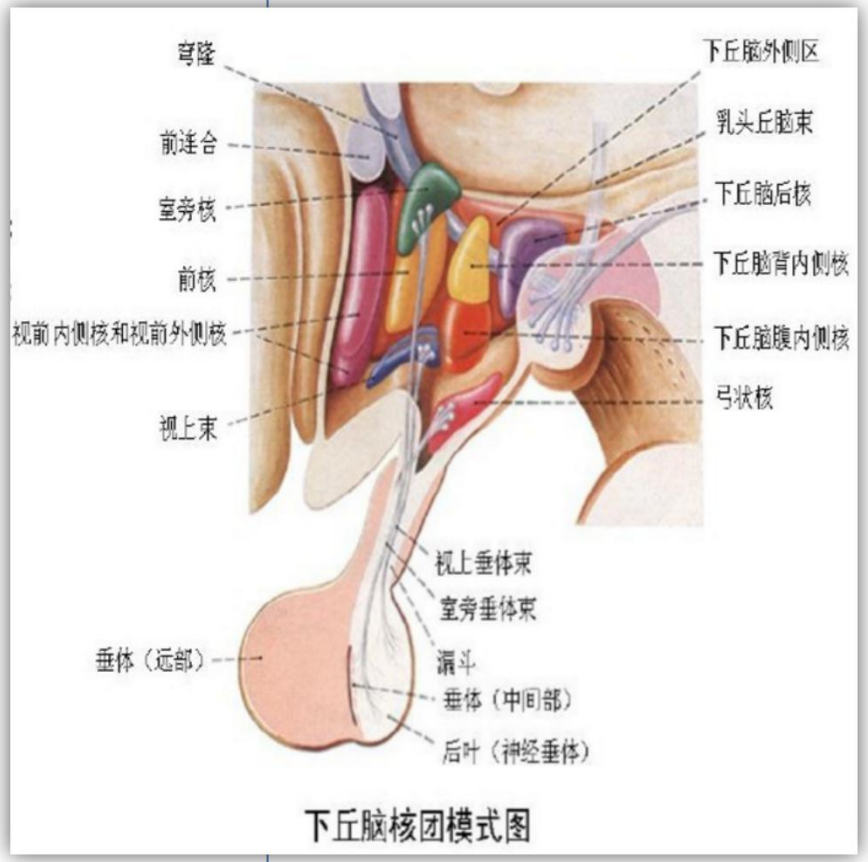
4 Effect of acute i.c.v. HD apelin injection on peripheral glucose metabolism in physiological and pathophysiological conditions

HFD
高糖高脂小鼠



ITT
胰岛素耐受实验

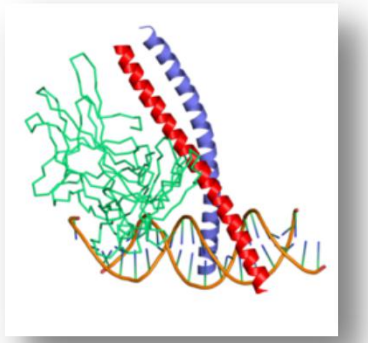
Figure 6, Duparc *et al.*



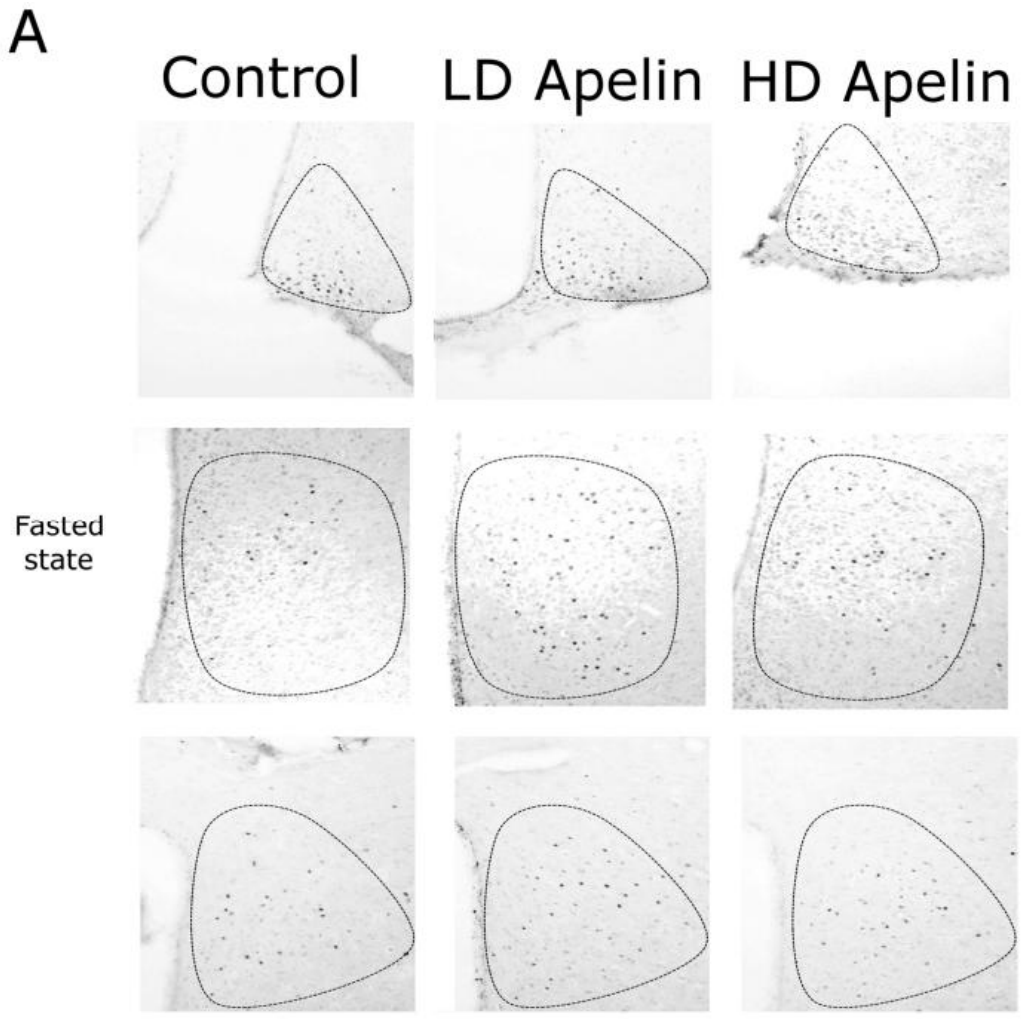
下丘脑核团模式图

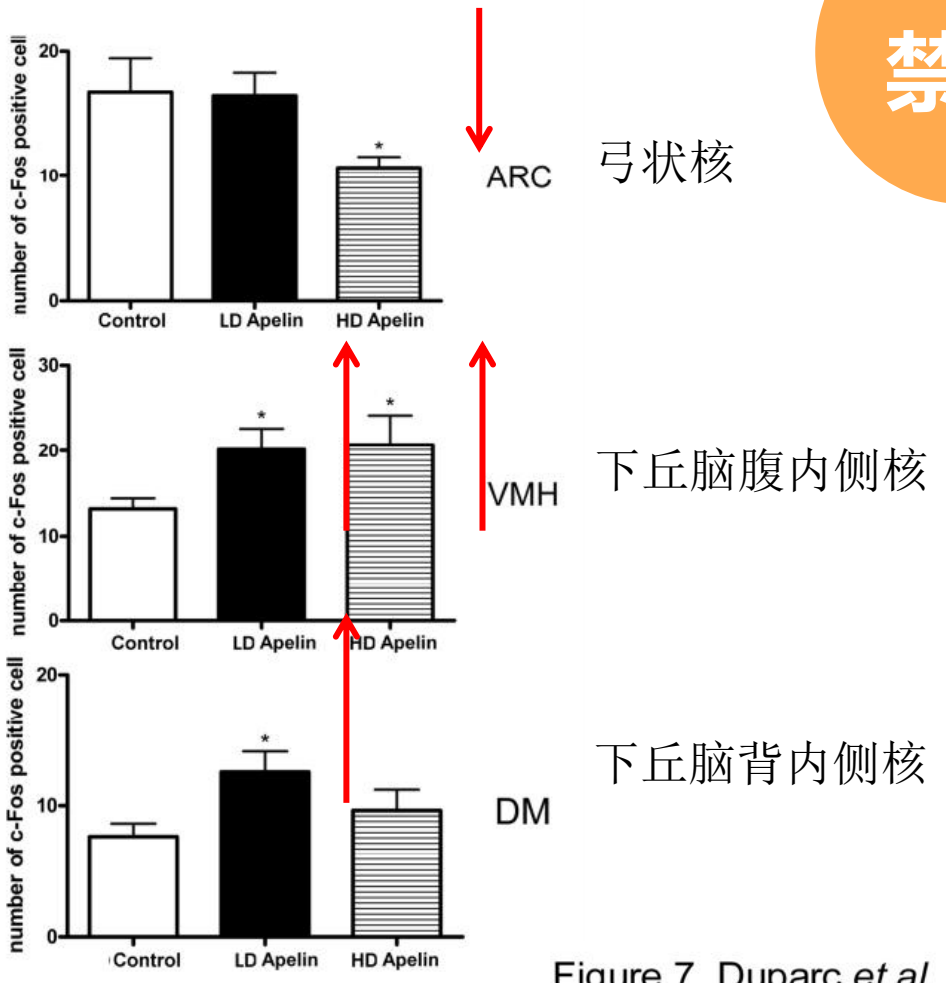
根据注射剂量和营养状态的不同，血糖/血液胰岛素对 I.C.V apelin产生不同的应答。提出一种可能：不同下丘脑区域是造成这些影响的原因。

FOS蛋白作为一类核蛋白转录因子，在调控细胞生长、分裂、增殖、分化乃至程序性死亡等方面具有重要的作用。FOS蛋白是神经元被刺激激活的一种标志。



5 Variation of c-Fos expression in the hypothalamus in response to acute apelin injection in physiological conditions

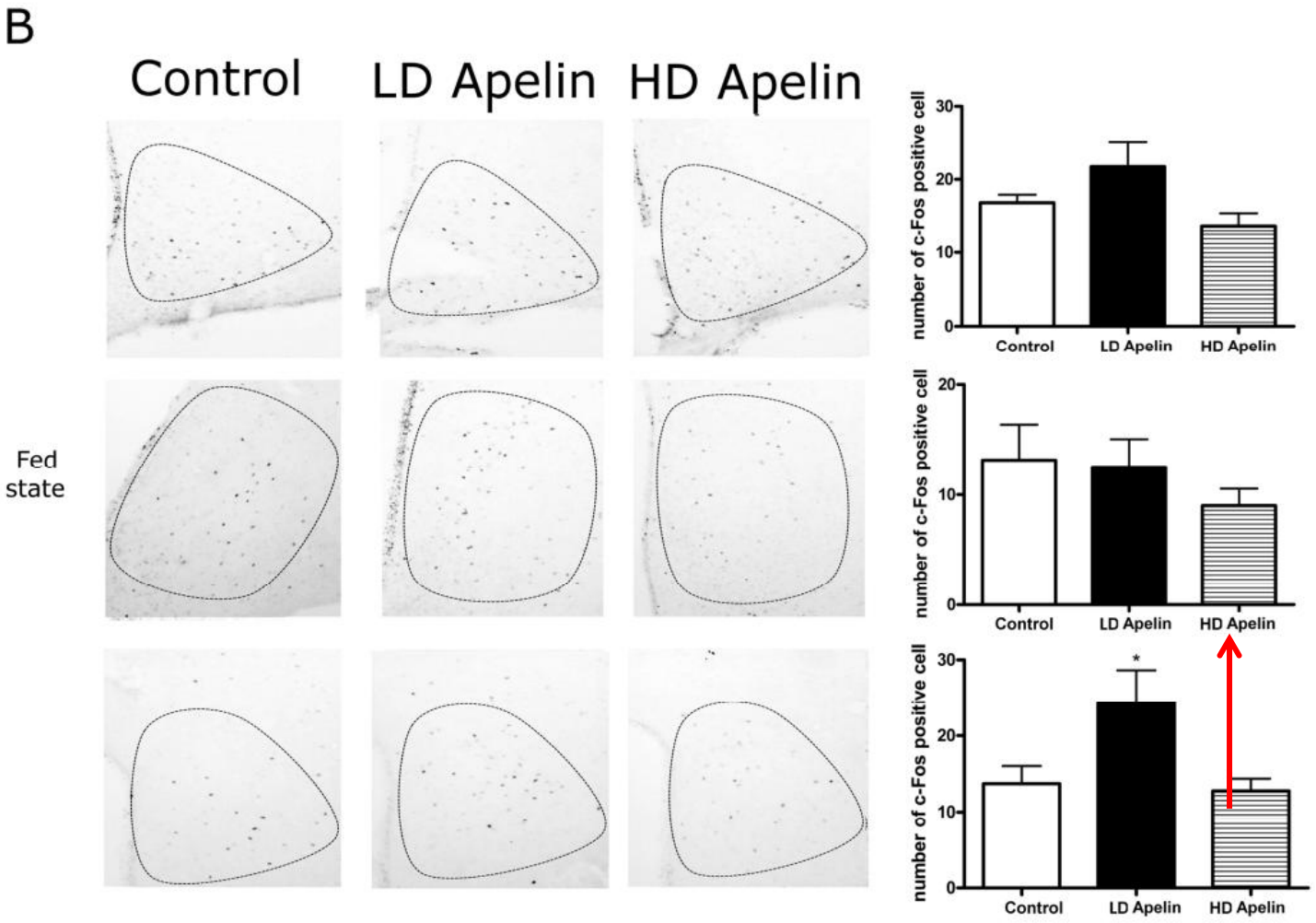




禁食

Figure 7, Duparc *et al.*

5 Variation of c-Fos expression in the hypothalamus in response to acute apelin injection in physiological conditions



喂食

ARC 下丘脑弓状核

VMH 下丘脑腹内侧核

DM 下丘脑背内侧核

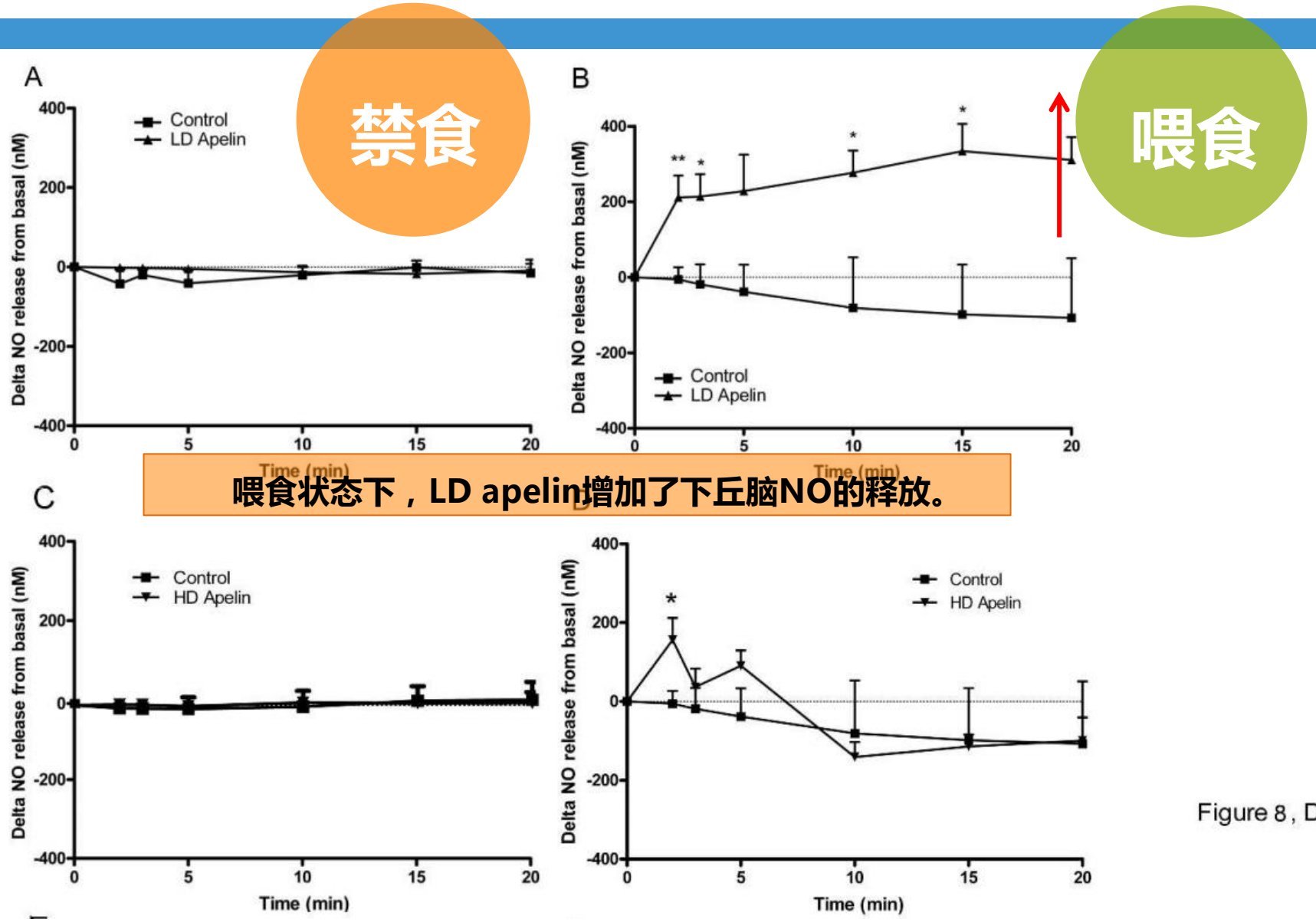
Figure 7, Duparc *et al.*

注射剂量和营养状态不同，下丘脑不同的部位被激活。说明：apelin是产生有益还是有害的影响可以通过不同区域的不同活化状态来解释。下丘脑apelin的高表达可能是糖尿病状态建立的关键因素。

6 Hypothalamic NO as a potential target to brain LD apelin

LD

HD



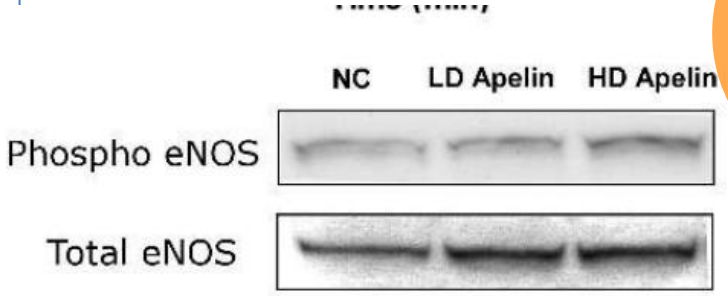
喂食状态下，LD apelin增加了下丘脑NO的释放。

Figure 8 , Duparc *et al.*

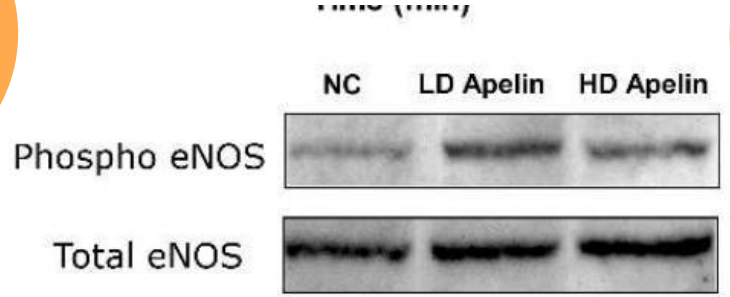
6 Hypothalamic NO as a potential target to brain LD apelin

E

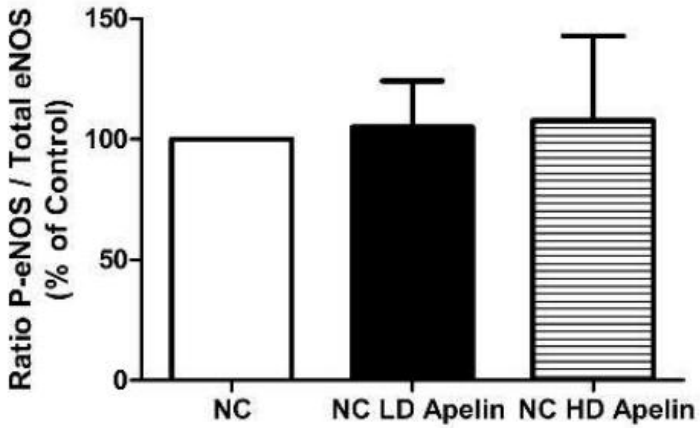
禁食



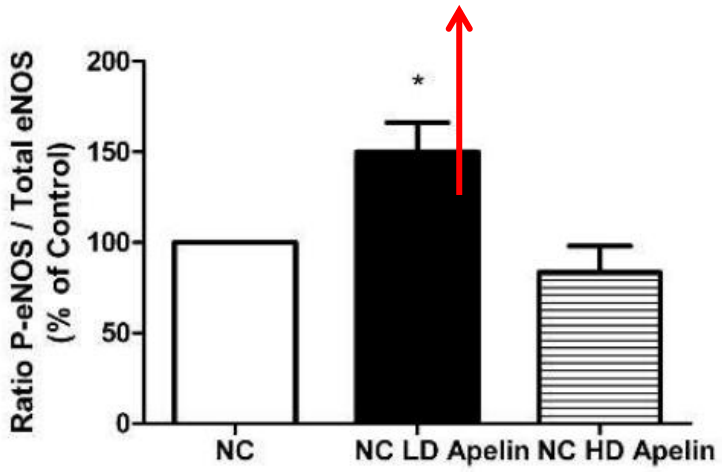
喂食



G



H



LD apelin引起的NO释放量的增加伴随着eNOS活性的增加。

6 Hypothalamic NO as a potential target to brain LD apelin

HFD
高糖高脂小鼠

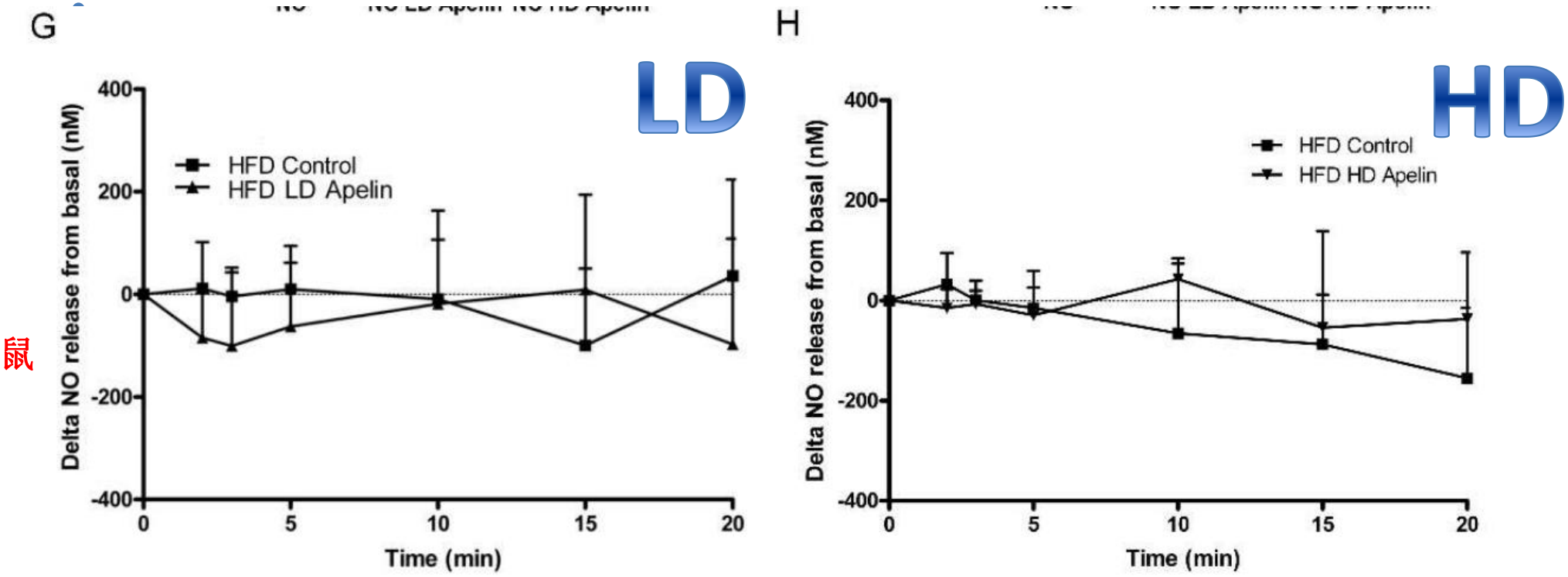
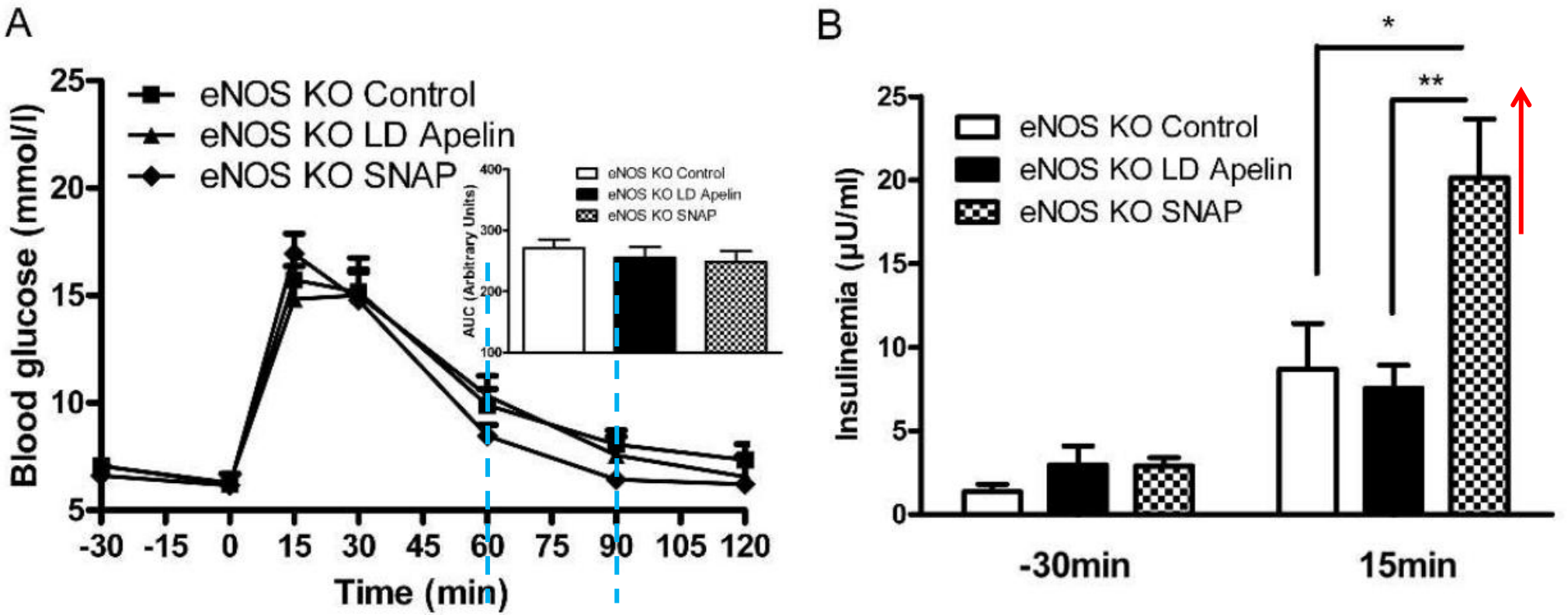


Figure 8 , Duparc *et al.*

在HFD小鼠中：1）LD apelin的作用受损。2）下丘脑eNOS途径被阻断。（数据未显示）说明肥胖和胰岛素抵抗状态扰乱了apelin-NO依赖的下丘脑反应。

7 Effect of acute i.c.v. LD or HD apelin on peripheral glucose metabolism in total eNOS KO and L-NMMA i.c.v. control treated mice

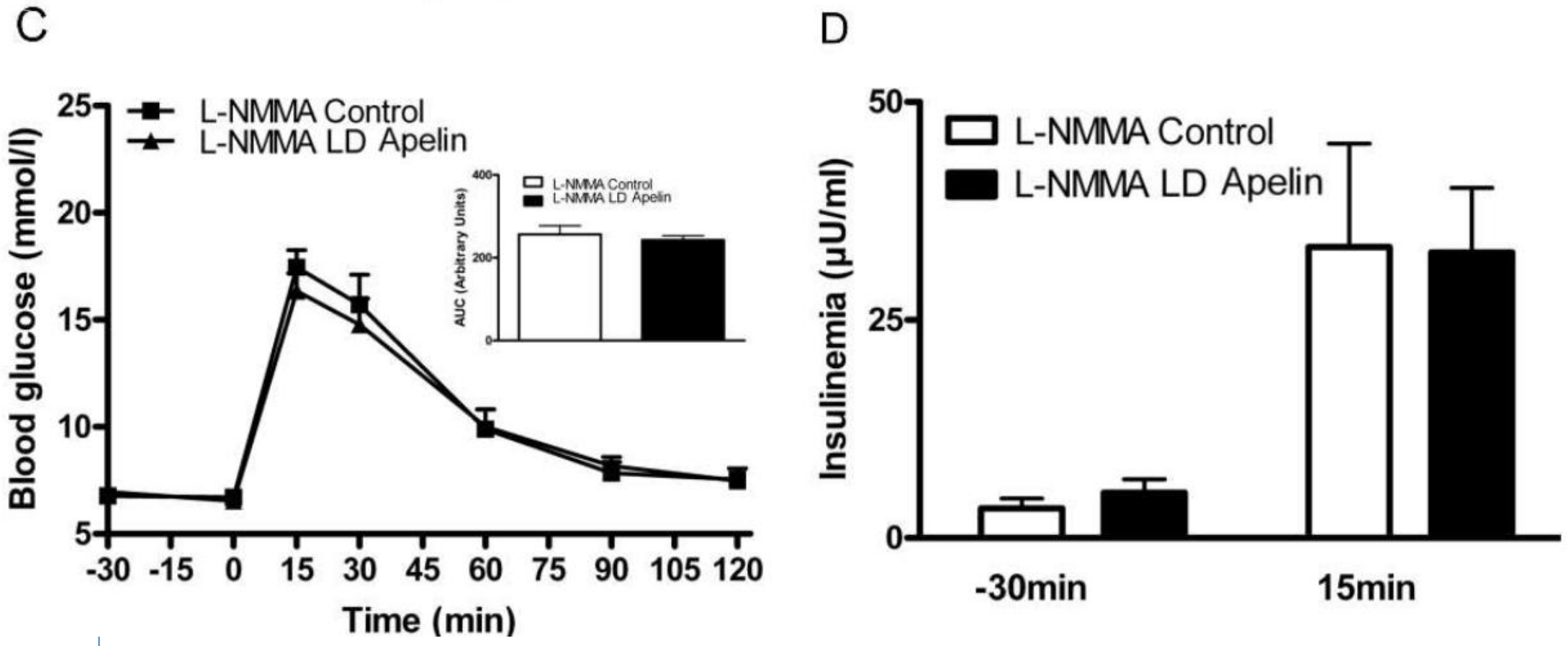


eNOS KO小鼠，LD apelin没有降低血糖。

NO供体显著增加了胰岛素的释放（血糖有轻微的下调）。

Figure 9, Duparc *et al.*

7 Effect of acute i.c.v. LD or HD apelin on peripheral glucose metabolism in total eNOS KO and L-NMMA i.c.v. control treated mice



L-NMMA阻断NO途径，LD apelin没有降低血糖。

Figure 9, Duparc *et al.*

7 Effect of acute i.c.v. LD or HD apelin on peripheral glucose metabolism in total eNOS KO and L-NMMA i.c.v. control treated mice

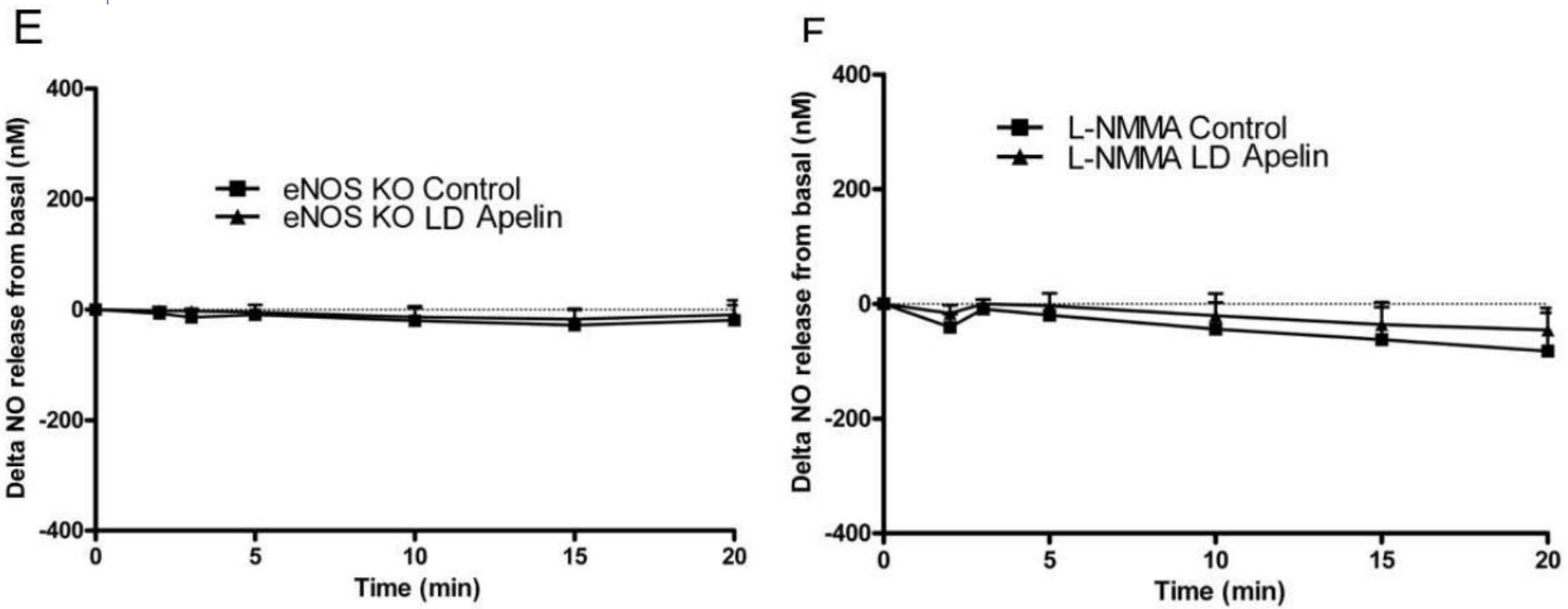


Figure 9, Duparc *et al.*

eNOS KO小鼠或用L-NMMA阻断NO途径，LD apelin不能增加NO的释放。

正常小鼠 喂食状态下，与对照相比，LD apelin 能够降低血糖，伴随NO释放的增加和eNOS 活性的增加，而阻断NO途径， LD apelin 则能够降低血糖。证明： LD apelin 能够降低血糖，是通过NO途径。

而对于HFD小鼠，肥胖和胰岛素抵抗状态阻断NO途径， LD apelin 则不能够降低血糖。

7 Effect of acute i.c.v. LD or HD apelin on peripheral glucose metabolism in total eNOS KO and L-NMMA i.c.v. control treated mice

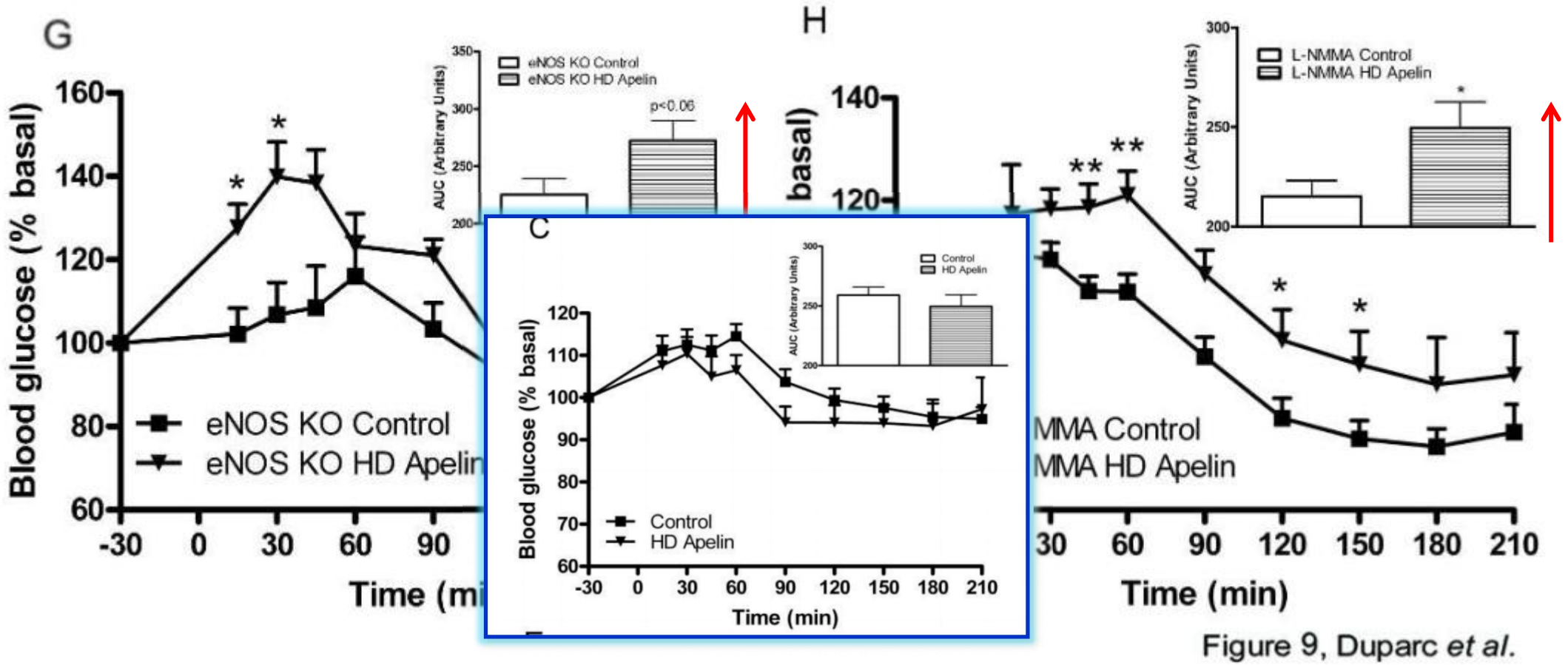


Figure 9, Duparc *et al.*

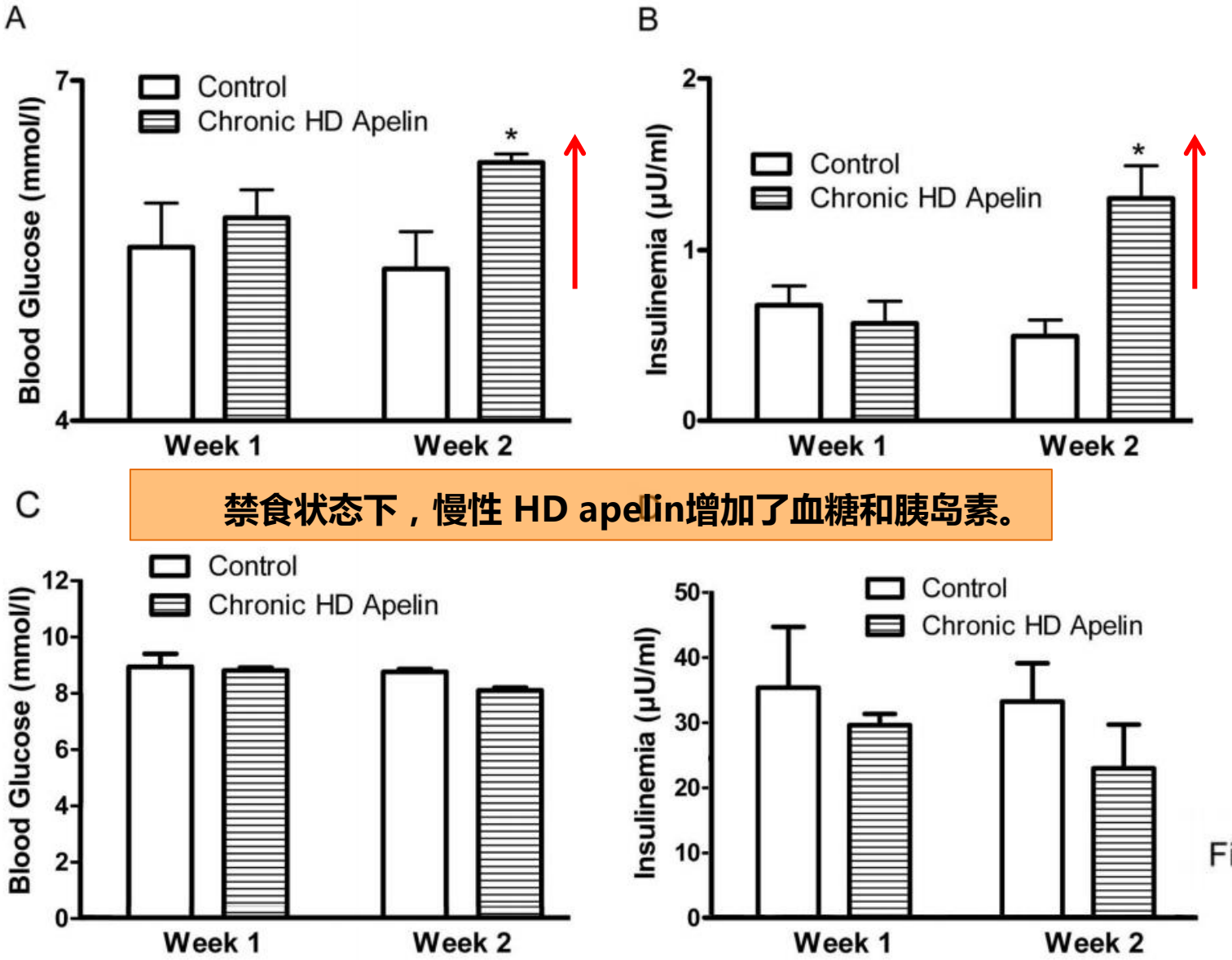
eNOS KO小鼠或用L-NMMA阻断NO途径，HD apelin升高了血糖。

说明

1、HD apelin升高血糖的作用是不依赖NO的，存在别的NO-非依赖性途径介导HD apelin 的作用。

2、在正常小鼠中，在喂食状态下，基础的下丘脑NO足以抵消HD apelin造成的高血糖。

8 Effect of i.c.v. chronic HD apelin injection on peripheral glucose metabolism in physiological conditions



禁食状态下，慢性 HD apelin增加了血糖和胰岛素。

禁食

喂食

Figure 10, Duparc *et al.*

8 Effect of i.c.v. chronic HD apelin injection on peripheral glucose metabolism in physiological conditions

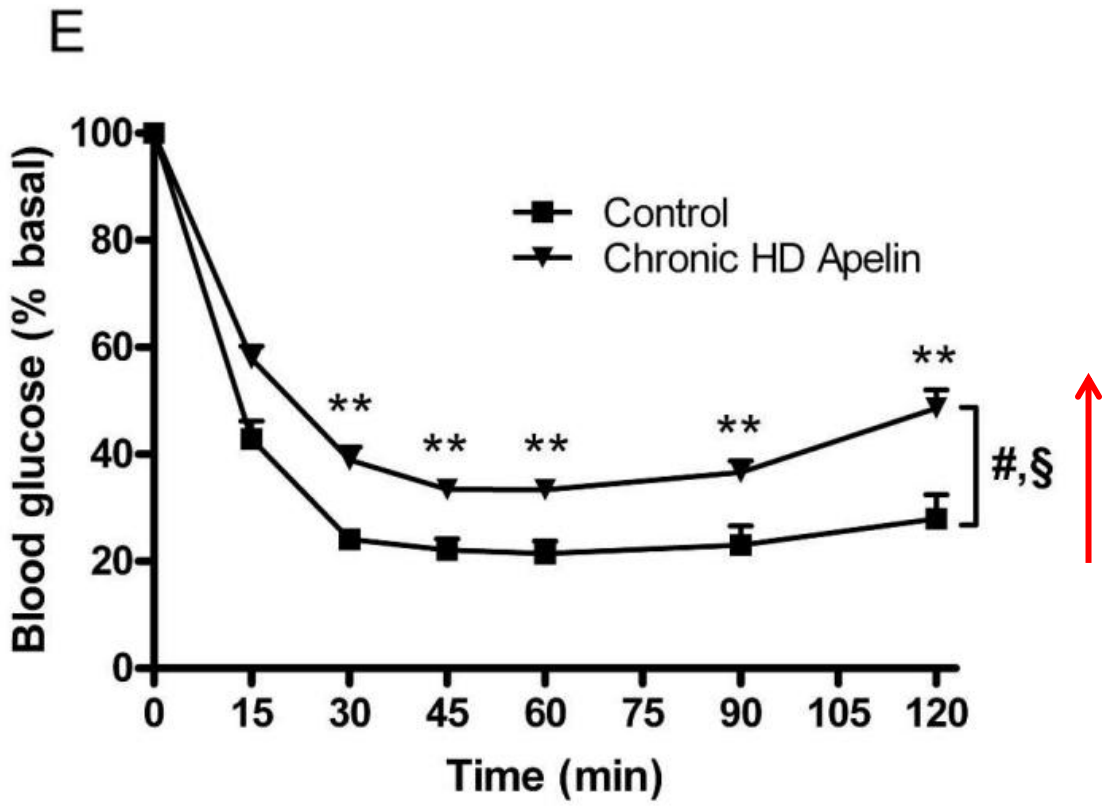


Figure 10, Duparc *et al.*

ITT
胰岛素耐受实验

慢性 HD apelin降低胰岛素耐量。

总结和讨论

饲喂状态下：

LD apelin → 血浆apelin增加 → NO（eNOS）→ 促进葡萄糖的吸收 → 葡萄糖稳态

维持

禁食状态下：大脑不能充分响应LD apelin。

其中一个解释是：下丘脑需要外周因子（包括葡萄糖and/or胰岛素）的注入才能充分的通过NO响应脑apelin。

营养素 → 血浆激素 → 下丘脑神经元活性（c-Fos表达）→ 响应LD apelin

总结和讨论

饲喂状态下：

破坏

HD apelin → 血浆apelin ↑ ✕ → NO（eNOS） → 降低葡萄糖的吸收 → 葡萄糖稳态

产生胰岛素抵抗

其中一个解释是：

HD apelin能够激活eNOS的抑制因子（如β-内啡肽，活性氧）。

禁食状态下：

破坏

HD apelin → 血浆apelin ↑ → 高血糖/胰岛素抵抗 → 葡萄糖稳态

其中一个解释是：HD apelin升高血糖不依赖NO途径，存在其他途径，如通过自主神经介导的肝糖原代谢。

VMH激活 → 肝糖原分解、糖异生 ↑ → 血糖 ↑

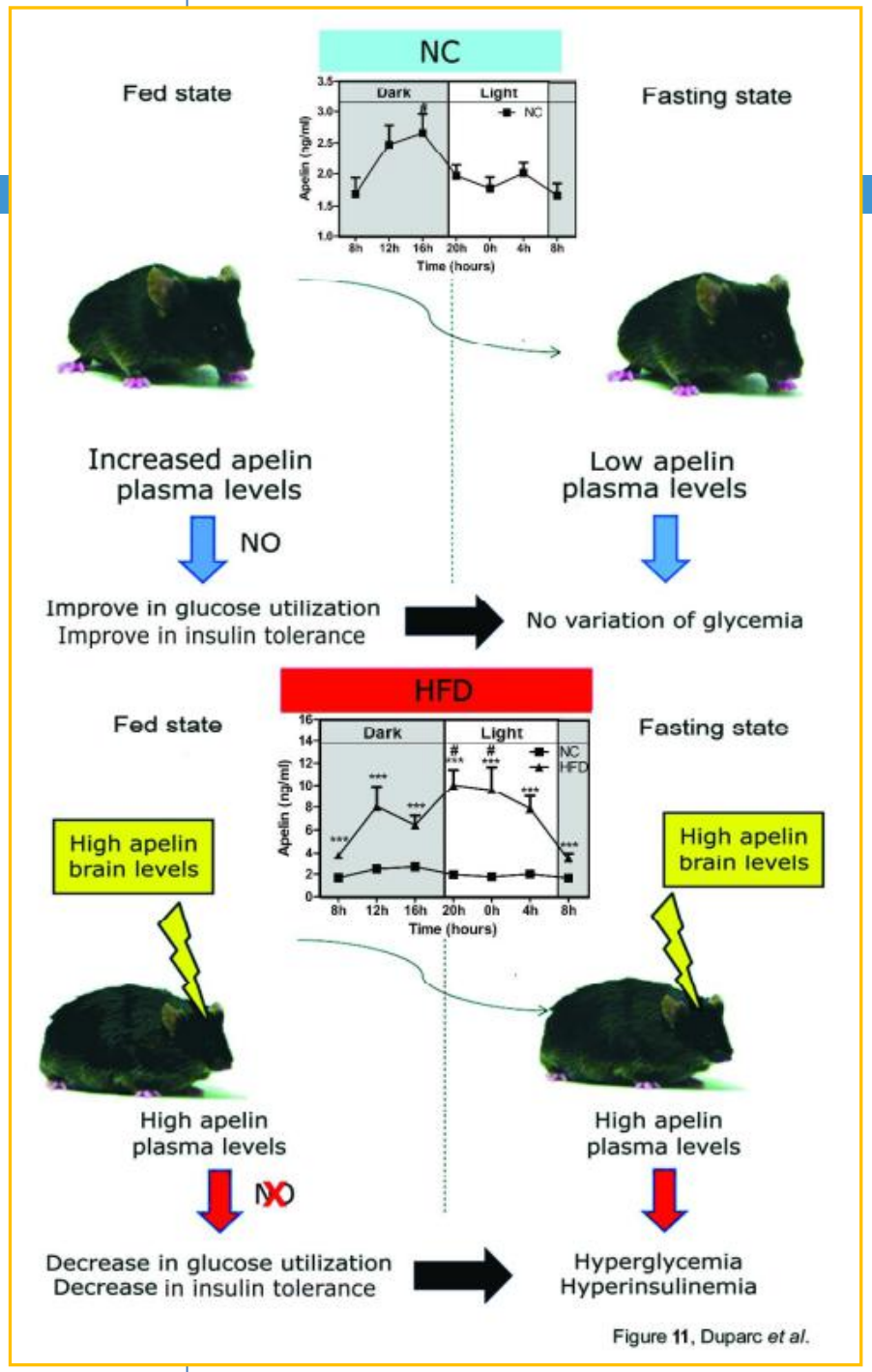


Figure 11, Duparc *et al.*

在生理状态下，血浆Apelin水平的升高对外周血糖会产生有利影响。如促进葡萄糖的吸收和改善胰岛素抵抗。

在病理状态下，血浆中很高的 Apelin水平可能会在大脑中产生有害的影响，在禁食的条件下导致高血糖和高胰岛素血症，提出apelin的一个新的角色，即介导从正常到糖尿病状态的转变。

愚人食盐

从前有一个愚笨的人，去亲戚家吃饭，主人在菜中加盐，菜都很美味可口。傻人以为盐既然那么好吃，回去每餐都买盐来吃好了，省得煮那么多菜。于是到了街上买了一大包的盐，回到家里急急打开，抓了一把放进口里，结果，他尝到的不是美味，而是又苦又涩吃了想吐的味道。这个故事告诉我们：一是看到事物内部的联系，不能单一形而上学的看问题，二是干任何事情都要有一个限度，恰到好处时美妙无比，一旦过头就会走向反面，哪怕是好事也会给弄得很糟。

天下之事皆然，过则非唯无益，反害之。

Thanks

感谢您的聆听

请提出宝贵意见！