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糖尿病大鼠 ghrelin 和 nesfatin-1 表达动力学及其调控 *

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摘要 目的：探讨 STZ 诱导糖尿病大鼠 ghrelin 和 nesfatin-1 动力学及分泌调节变化。**方法：**STZ 诱导糖尿病大鼠模型；采用葡糖糖脱氢酶分析法测量血浆葡萄糖水平；免疫放射分析检测血浆 ghrelin、nesfatin-1、胰岛素、胰岛素样生长因子 1(IGF-1)、生长激素(GH)含量；采用 real-time PCR 检测 ghrelin mRNA 水平变化；免疫组化观察 ghrelin 和 nesfatin-1 免疫活性细胞数量。**结果：**糖尿病大鼠体重显著降低(t=23.16，P＜0.01)，血糖水平显著升高(t=22.55，P＜0.01)，血浆胰岛素和 IGF-1 水平显著降低(t=6.50，t=24.13，P＜0.01)，但 GH 水平显著升高(t=3.30，P＜0.05)。糖尿病大鼠血浆总 ghrelin（t=7.03，P＜0.01）和活性 ghrelin(t=3.33，P＜0.05)水平均显著升高，血浆 nesfatin-1 水平则显著降低（t=6.24，P＜0.01）；糖尿病大鼠血浆总 ghrelin 与 GH(r=0.81，P＜0.01) 和 IGF-1 水平(r=-0.58，P＜0.01)呈显著相关性；与对照组大鼠相比，糖尿病大鼠胃总 ghrelin(t=16.86，P＜0.01)和活性 ghrelin(t=3.30，P＜0.05)水平均显著降低；而胃 nesfatin-1（t=7.93，P＜0.01)水平则显著升高。胃总 ghrelin 水平与血浆 IGF-1 水平呈明显相关性(r=0.65，P<0.01)；与对照组大鼠相比，糖尿病大鼠胃 ghrelin mRNA 表达水平显著升高(t=16.8，P＜0.01)，胃底 ghrelin 免疫活性细胞数量显著减少（t=3.98，P＜0.01）；实验中给予大鼠自由饮食，糖尿病大鼠血浆总 ghrelin 水平显著增加(t=7.53，P＜0.01)，nesfatin-1 水平显著降低 (t=5.46，P＜0.01)。糖尿病大鼠注射胰岛素后，可使增加的 ghrelin 水平 (t=1.76，P=0.11)和降低的 nesfatin-1 水平接近正常 (t=1.96，P=0.06)；且胰岛素可显著反转糖尿病大鼠胃总 ghrelin（t=8.54，P＜0.01)和 nesfatin-1 水平（t=2.42，P＜0.05)；以及注射胰岛素后，糖尿病大鼠胃底 ghrelin 细胞显著增加，nesfatin-1 细胞明显减少（t=3.21，t=2.59，P＜0.05)。**结论：**Ghrelin 或 nesfatin-1 参与糖尿病大鼠能量平衡调控。

关键词：Ghrelin；Nesfatin-1；糖尿病；胃；生长激素

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Dynamics Expression and Regulation of Ghrelin and Nesfatin-1 in Diabetic Rats*

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ABSTRACT Objective: To study the changes of ghrelin and nesfatin-1 dynamics and secretory regulation in rats with STZ-induced diabetes. **Methods:** Diabetes was induced by intraperitoneal injection of streptozotocin (STZ). Serum glucose were measured by the glucose dehydrogenase, while the levels of ghrelin, nesfatin-1, insulin, IGF-1 and GH were detected by RIA. Preproghrelin mRNA level was tested using real-time RT-PCR and the ghrelin and nesfatin-1-immunoreactive cells were observed using immunohistochemistry. **Results:** The weight of diabetic rats decreased significantly (t=23.16, P<0.01), the glucose was increased significantly (t=22.55, P<0.01), plasma insulin and IGF-1 level was decreased significantly (t=6.50, t=24.13, P<0.01), but the GH level was increased significantly (t=3.30, P<0.05). The level of total (t=7.03, P＜0.01) and active (t=3.33, P<0.05) plasma ghrelin of the diabetic rats was increased significantly, while the plasma nesfatin-1 level was decreased (t=6.24, P＜0.01), compared with that of the control group. The total plasma ghrelin level of the diabetic rats correlated significantly with their serum GH level (r=0.81, P<0.01) and with their serum IGF-1 level (r=-0.58, P<0.01). The gastric total (t=16.86, P<0.01) and active (t=3.30, P<0.05) ghrelin level of the diabetic rats was decreased significantly, while the gastric nesfatin-1 levels was increased(t=7.93, P＜0.01) compared with that in the control group, and their gastric total ghrelin level correlated significantly with their serum IGF-1 level (r=0.65, P<0.01). The preproghrelin mRNA expression in the stomach was increased significantly (t=16.8, P<0.01) in the diabetic rats compared with the control and the number of ghrelin-immunoreactive cells in the gastric fundus of the diabetic rats was decreased significantly (t=3.98, P<0.01). Giving rats free diet in the experiments, the total plasma ghrelin level in diabetic rats was increased significantly (t=7.53, P<0.01), nesfatin-1 level

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significantly reduced (t=5.46, P<0.01). After insulin injections, the increasing level of ghrelin (t=1.76, P=0.11) and reducing level of nesfatin-1 in diabetic rats was close to normal (t=1.96, P=0.06). Insulin significantly reversed the total gastric ghrelin (t=8.54, P<0.01) and nesfatin-1 level (t=2.42, P<0.05) of diabetes rats. After injection of insulin, ghrelin cells were increased significantly, while nesfatin-1 cells were decreased significantly (t=3.21, t=2.59, P<0.05) in stomach fundus of diabetic rats. **Conclusions:** Ghrelin or nesfatin-1 may participate in the regulation of energy balance in diabetic rats.

Key words: Ghrelin; Nesfatin-1; Diabetes; Stomach; Growth hormone

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前言

Ghrelin 是从人类和大鼠胃中分离的一种新型生长激素促分泌素 [1]，在其第 3 位丝氨酸残基上有特异性 N 端辛酰基修饰，对 ghrelin 的生理特性至关重要。Ghrelin 的主要生理功能是促进人类及啮齿类动物生长激素分泌，促进摄食[2]及胃运动[3]。负能量平衡状态比如饥饿或恶病质、神经性厌食症所致的低体重可使血浆 ghrelin 水平增加[4-8]。许多组织可表达前体 ghrelin mRNA[9]，其中胃底表达水平最高[10]。Nesfatin-1 是 2006 年发现的一种新型厌食肽[11]，在中枢、外周胃肠、胰岛等部位均有表达，具有减少摄食、抑制胃肠运动等作用。据报道胃底 A 样细胞可分泌 ghrelin 和 nesfatin-1。

链脲佐菌素（STZ）是一种抗生素，可通过破坏胰腺胰岛内分泌细胞诱导糖尿病生成。STZ 诱导糖尿病大鼠可出现体重降低、多食、血糖升高、低胰岛素血症[12,13]。至今还没有报道 STZ 诱导胰岛素依赖型糖尿病大鼠 ghrelin 和 nesfatin-1 的动力学变化。本文应用免疫放射分析法（RIA）、实时酶联反应法（RT-PCR）及免疫组织化学技术等方法，探讨 STZ 诱导糖尿病大鼠 ghrelin 和 nesfatin-1 动力学及分泌调节变化。

1 材料和方法

1.1 实验动物

8 周龄雄性 Wistar 大鼠 45 只，随机分为 3 组：对照组(n=15)，腹腔注射生理盐水；STZ 诱导糖尿病大鼠组(n=15)，腹腔注射 STZ (60 mg/kg) 诱导糖尿病；胰岛素处理糖尿病大鼠组(n=15)，在注射 STZ 一周后给予皮下注射中性鱼精蛋白人胰岛素(7 U/ 只)。所有动物自由饮水和进食，4 周后大鼠禁食 16 h，乙醚麻醉处死。分离大鼠胃、十二指肠和上端结肠，生理盐水冲洗。

1.2 血液及相关激素分析

采用葡糖糖脱氢酶分析法测量血浆葡萄糖水平，放射免疫分析（RIA）参照文献[14,15] 进行，检测血浆胰岛素、胰岛素样生长因子 1（IGF-1）、生长激素（GH）以及血浆和胃组织 ghrelin、nesfatin-1 水平。

1.3 Real-time PCR

使用 Tri-RNA 试剂盒提取下丘脑组织总 RNA，用 1 μg 总 RNA 和寡核苷酸 dT 引物合成 cDNA。cDNA 与 SYBR Premix Ex TaqII 和特定引物混合，进行 RT-PCR：95° C 3 s，60° C 30 s，50 个循环。Ghrelin 引物：5′ -GGA ATC CAA GAA GCC ACC AGC-3′ ；5′ -GCT CCT GACAGC TTG ATG CCA-3′ 。Ghrelin 探针序列：5′ -FAM-AAC TGC AGC CAC GAG CTC TGG AAG GC-TAMRA-3′ 。内源性对照 GAPDH 引物：5′ -TTC AAC GGC ACA GTC AAG GC-3′ ；5′ -GCC TTC TCC ATG GTG GTG AAG-3′ 。GAPDH 探针序列：5′ -FAM-CCC ATC ACC ATC TTC CAG GAG CGA GA-TAMRA-3′ 。

1.4 免疫组织化学染色

组织样本用 10%福尔马林固定，石蜡包埋，连续冠状切片，厚度为 4-6 μm。切片 70℃ 加热 10 min，之后依次浸泡于下述溶液中：二甲苯（5 min），96%乙醇（3 min），90%乙醇（3 min），双蒸水（3 min）。切片经 nesfatin-1（1:2000，美国 Phoenix 公司）或 ghrelin（1:10000，美国 sigma 公司）一抗孵育，4℃ 过夜[11]，磷酸盐缓冲液清洗后，用羊抗兔生物素 Ig G 孵育 4℃ 过夜[11]。切片经卵白素 - 生物素 - 过氧化物酶复合物着色 10 min[11]，梯度乙醇溶液中脱水，苏木紫中性染色。光镜下观察。免疫活性细胞密度(D)用以下公式计算：

$D=(Ng/Nt)\times 100\%$

Ng：一定区域免疫活性细胞的数量；Nt：免疫活性细胞的总数量。

1.5 统计学分析

所有数据均用（$\bar{x}\pm s$）表示，多组间均数比较采用单因素方差分析，两组间均数比较采用 t 检验，Real-time PCR 结果分析采用△Ct 值表示，即检测待测基因 cDNA 进入 PCR 指数增长期的起始点即循环阈值（cycle threshold, Ct），以同一样本中神经肽和看家基因 β -actin 之间的 Ct 差值（△Ct），即 $\triangle Ct=Ct_{ghrelin}-Ct_{\beta\text{-}actin}$ 进行组间资料的 t 检验（Prism 3.0 统计软件），P<0.05 为差异有显著统计学意义。

2 结果

与对照组相比，糖尿病大鼠体重显著降低 (t=23.16，P< 0.01; 表 1)，糖尿病大鼠胃湿重与对照组大鼠比较无明显差异（t=0.68，P>0.05）。血糖水平显著升高(t=22.55，P< 0.01)，血浆胰岛素和 IGF-1 水平显著降低 (t=6.50，t=24.13，P< 0.01)，但 GH 水平显著升高(t=3.30，P< 0.05) 。

与对照组大鼠相比，糖尿病大鼠血浆总 ghrelin（t=7.03，P< 0.01）和活性 ghrelin (t=3.13，P< 0.05) 水平均显著升高，而 nesfatin-1（t=6.24，P< 0.01）水平则显著降低（表 2）。糖尿病大鼠血浆总 ghrelin 水平与 GH 水平 (r=0.81，P< 0.01) 和 IGF-1 水平 (r=-0.58，P< 0.01) 呈显著相关性（图 1A,B）。与对照组大鼠相比，糖尿病大鼠胃总 ghrelin (t=16.86，P< 0.01) 和活性 ghrelin (t=3.30，P< 0.05) 含量均显著降低，而胃 nesfatin-1 含量则显著升高（t=7.93，P< 0.01，表 2），胃总 ghrelin 水平与血浆 IGF-1 水平呈明显相关性 (r=0.65，P< 0.01，图 1C)。

表 1 糖尿病大鼠体重、血糖、胃湿重及血浆胰岛素和 GH 水平($\bar{x}\pm s$)

Table 1 The levels of body weight, gastric weight, serum glucose, serum insulin, serum IGF-1 and serum GH in STZ-induced diabetic rats($\bar{x}\pm s$)

	Body weight (g)	Gastric weight(mg)	Glucose (mg/dl)	Insulin (ng/mL)	GH (ng/mL)	IGF-1 (ng/mL)
Control	353.4± 16.0	2145.6± 81.2	136.5± 4.1	0.27± 0.08	6.63± 0.24	1478.6± 137.6
Diabetic	207.5± 12.2**	2160.5± 85.0	451.2± 41.5**	0.08± 0.05**	35.23± 13.24*	449.5± 32.5**

注:*P<0.05,**P<0.01,与对照组相比。

Notes: *P<0.05, **P<0.01 vs. control group.

表 2 糖尿病大鼠血浆和胃组织 ghrelin 和 nesfatin-1 的表达($\bar{x}\pm s$)

Table 2 The level of plasma ghrelin, nesfatin-1 and gastric ghrelin, nesfatin-1in STZ-induced diabetic rats ($\bar{x}\pm s$)

Group	Total Ghrelin		Active Ghrelin		Nesfatin-1	
	Plasma (fmol/mL)	Gastric (fmol/mg)	Plasma (fmol/mL)	Gastric (fmol/mg)	Plasma (fmol/mL)	Gastric (fmol/mg)
Control (n=15)	615.2± 72.3	1758.8± 184.3	76.2± 25.6	337.5± 25.8	357.8± 53.1	1132.5± 96.5
Diabetic (n=15)	1123.4± 196.6**	1007.5± 195.2**	120.2± 38.5*	205.2± 36.5**	216.2± 48.3*	1385.5± 103.3*

注:*P<0.05,**P<0.01,与对照组相比。

Notes: *P<0.05, **P<0.01 vs. control group.

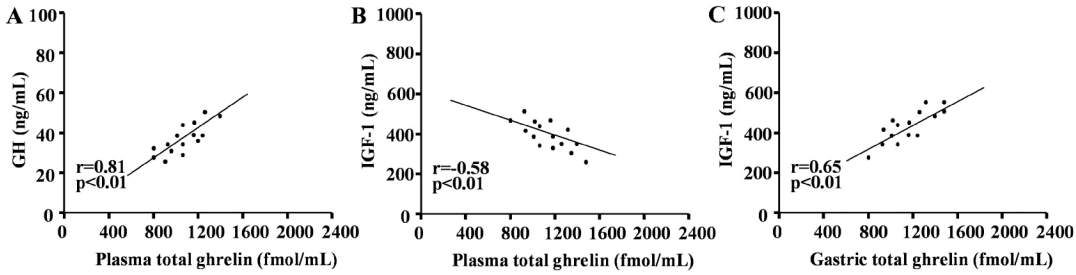

图 1 Ghrelin 与 GH,IGH-1 水平的相关性分析

Fig. 1 The correlation between ghrelin and GH, IGH-1

注:A:血浆总 ghrelin 与 GH 水平的相关性分析;B:血浆总 ghrelin 与 IGF-1 水平的相关性分析;C:胃总 ghrelin 与 IGF-1 水平的相关性分析。

Note: A: The correlation between plasma total ghrelin and GH; B: The correlation between plasma total ghrelin and IGF-1; C: The correlation between gastric total ghrelin and IGF-1.

与对照组相比，糖尿病大鼠胃 ghrelin mRNA 表达水平显著升高(t=16.8,P＜0.01;图 2a),但十二指肠和结肠 ghrelin mRNA 表达水平与对照组无显著差异(t=1.24, P>0.05)。与对照组相比，糖尿病大鼠胃底 ghrelin 免疫活性细胞数量显著减少(t=3.98,P<0.01;图 2b),胃底 ghrelin 免疫活性细胞与细胞总数比例减小,提示糖尿病大鼠胃非活性细胞增加,但与对照组相比差异不明显(t=1.64,P=0.11;图 2c)

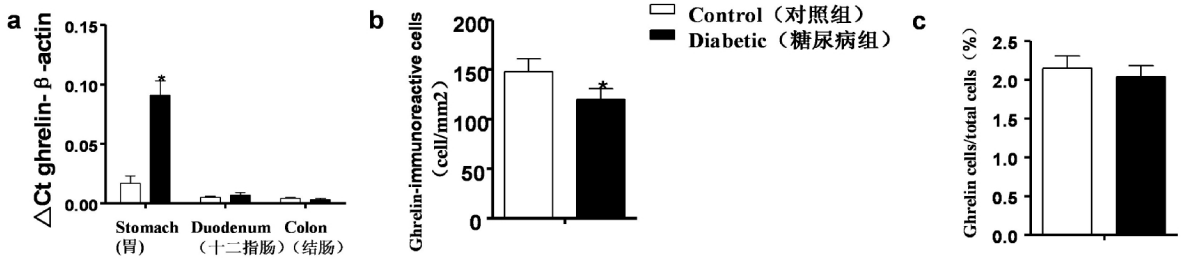

图 2 糖尿病大鼠 ghrelin mRNA 和 ghrelin 免疫反应细胞的表达

Fig. 2 The expression of preproghrelin mRNA and ghrelin-immunoreactive cells in STZ-induced diabetic rats

注:a:胃、十二指肠和结肠中 ghrelin mRNA 的表达(ghrelin/β -actin);b:糖尿病大鼠 ghrelin 免疫活性细胞数量;c:胃底 ghrelin 免疫活性细胞与细胞总数比例。*P<0.01,与对照组相比

Note: a: the expression of ghrelin mRNA in stomach, duodenum and colon(ghrelin/β -actin);b:the number of ghrelin-immunoreactive cells of diabetic rats; c: the ratio of ghrelin-immunoreactive cells in total cells in fundus of stomach. *P<0.01 vs. control group.

实验中给予大鼠自由饮食，糖尿病大鼠血浆总 ghrelin 水平显著增加 (t=7.53,P＜0.01; 表 3),nesfatin-1 水平显著降低(t=5.46,P＜0.01)。胰岛素处理后可使增加的 ghrelin 水平降低接近正常 (t=1.76,P=0.11)，而使降低的 nesfatin-1 水平升高(t=1.96,P=0.06)。与对照组相比,糖尿病大鼠胃总 ghrelin 水平降低(t=5.14,P＜0.01),而 nesfatin-1 水平显著升高(t=5.37,P＜0.01),给予胰岛素可显著反转 ghrelin(t=8.54,P＜0.01; 表 3)和 nesfatin-1 水平(t=2.42,P＜0.05)。在正常大鼠、糖尿病大鼠和胰岛素处理糖尿病大鼠之间，胃内活性 ghrelin 与总 ghrelin 的比率无显著差异(t=1.12,P>0.05)。与正常大鼠比较,糖尿病大鼠胃底 ghrelin 免疫活性细胞数显著减少 (t=3.56,P＜0.01),nesfatin-1 免疫活性细胞数量明显增加(t=3.56,P＜0.01),胰岛素处理后可显著改善这一状况(t=3.21,t=2.59,P＜0.05; 表 3)。

表 3 胰岛素对糖尿病大鼠 ghrelin 和 nesfatin-1 表达的影响($\bar{x}\pm s$)

Table 3 The effects of insulin on expression of ghrelin and nesfatin-1 in STZ-induced diabetic rats ($\bar{x}\pm s$)

Group	Plasma total ghrelin (fmol/mL)	Gastric total ghrelin (fmol/mg)	Ghrelin Cells (Cell/mm^2)	Plasma nesfatin-1 (fmol/mL)	Gastric nesfatin-1 (fmol/mg)	Nesfatin-1 Cells (Cell/mm^2)
Control (n=15)	492.1± 43.2	2238.1± 197.5	96.2± 12.5	275.5± 41.3	1252.3± 114.0	48.5± 9.6
Diabetic (n=15)	714.4± 58.4**	1652.1± 158.2**	61.7± 10.3**	182.6± 35.8**	1537.4± 123.1**	64.4± 11.3**
Diabetic+INS (n=15)	655.3± 52.5	2690.5± 204.5##	108.2± 22.5#	214.4± 38.6	1406.2± 119.2#	50.1± 6.5

注:P<0.01,与对照组相比;#P<0.05,##P<0.01,与糖尿病组相比。**

Notes: **P<0.01 vs. control group; #P<0.05, ##P<0.01 vs. diabetic group.

3 讨论

STZ 处理大鼠体重和血浆胰岛素水平显著降低,血糖水平显著升高。这种观点与之前研究结果一致 [13]，提示本实验中 STZ 诱导糖尿病大鼠模型成功。大鼠给予外源性 ghrelin 可增加血浆 GH 水平 [16],STZ 诱导的糖尿病大鼠血浆 GH 水平与 ghrelin 水平有显著相关性,与在恶病质和慢性心脏病中研究相符[6]。STZ 诱导糖尿病大鼠研究结果显示,血浆 ghrelin 水平升高,胃 ghrelin mRNA 表达水平降低,胃底 ghrelin 水平与 ghrelin 免疫活性细胞数量显著降低,而血浆 nesfatin-1 水平显著降低，胃 nesfatin-1 水平显著升高。有文献报道，十二指肠有 ghrelin、ghrelin mRNA 表达,ghrelin 免疫活性细胞数量也较多[11,17,18]，本研究显示结肠和十二指肠 ghrelin mRNA 表达水平无显著差异,提示,ghrelin 主要在胃中产生。且有报道 nesfatin-1 在胃组织中的表达是脑的 10 倍。提示糖尿病大鼠胃内 ghrelin 水平降低,nesfatin-1 水平升高可能与血浆 ghrelin 水平的升高,nesfatin-1 水平的降低有重要关联。

Ghrelin 和 nesfatin-1 均可由胃 X/A 样细胞分泌[19]。本研究发现,胰岛素处理大鼠可逆转胃底 ghrelin 和 nesfatin-1 免疫活性细胞数量的改变,提示胃 A 样细胞中 ghrelin 或 nesfatin-1 标记细胞数量改变,而不是 A 样细胞分泌 ghrelin 或 nesfatin-1 变化。糖尿病大鼠血浆 ghrelin 水平升高,胃 ghrelin 水平及 ghrelin 免疫活性细胞数量降低可能归因于胃 ghrelin 释放入血增多相关。糖尿病大鼠体重显著降低,胰岛素处理后可逆转糖尿病大鼠 ghrelin 或 nesfatin-1 动力学改变,这些改变可能导致负能量平衡,如体重降低,而不是直接逆转 STZ 对胃粘膜毒性作用。但对刺激肽类合成或分泌的信号通路机制还不清楚。

Ghrelin 是一种具有多重生理功能的脑肠肽，研究显示，ghrelin 与 IGF-1 相似具有双重调控代谢和生长的作用[20]。本研究结果发现，血浆 IGF-1 水平与血浆和胃总 ghrelin 水平有显著相关性,血浆 IGF-1 水平的降低可能参与这一调控机制。另外，负能量平衡状态可能引起胃 ghrelin mRNA 表达水平补偿性上调,使 ghrelin 合成分泌增加。

总之,STZ 诱导糖尿病大鼠胃 ghrelin 分泌入血增加,提示,在 STZ 处理大鼠,负能量平衡可能导致 ghrelin mRNA 水平上调,从而刺激胃 ghrelin 前体细胞合成分泌 ghrelin,而胃 nesfatin-1 水平升高可能与糖尿病胃轻瘫的发生发展有相关性。

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