孕期咖啡因暴露所致子代胰腺发育受限及糖代谢异常的宫内编程机制

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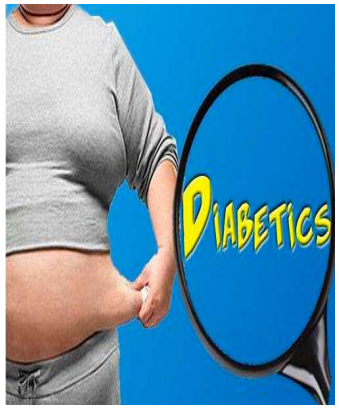
提纲

1. 引言
2. 研究内容
3. 全文结论

1. 糖尿病的发育起源



糖尿病（DM）

慢性血糖升高为特征
胰岛素分泌或作用缺陷所致
发病率逐年升高
以II型(T2DM)为主

Yang, S.H. et al. N Engl J Med,2010

Barker DJ. *Diabetologia* 1993

Low birth weight linked to diabetes

ANISHA FRANCIS

CHENNAI

June 18: Babies born with lower birth weight than normal are prone to developing diabetes in their later years, just those who are born overweight, a new study has found.

Babies weighing less than 2.5 kg at birth tend to put on weight quickly during the first three months of their life. This rapid weight gain adversely affects the glucose metabolism during adolescence, predisposing the children to diabetes and metabolic syndrome, the study published in Pubmed has revealed.

Nearly 25 per cent of babies born in Tamil Nadu have low birth weight.

Rapid weight gain is only one of the factors that predispose underweight newborns to diabetes and hypertension, says Deepa Hariharan, neonatologist at Surya Hospital here.

"When full-term babies are born with low birth weight, it means that their insulin mechanism is defective, because insulin is the hormone responsible for the growth of the fetus inside the womb," explains Dr Hariharan, who routinely treats infants weighing less than 1 kg at the neonatal ICU.

"While big 'sumo' babies weighing over 4.5 kilos, usually born to overweight mothers with diabetes, are likely to develop the ailment in adolescence, newborns with intra uterine growth retardation are equally at risk because of this insulin deficiency," she said.

Underweight infants are likely to be pumped with supplementary formula, making them rapidly put on weight and putting a strain on their already abnormal pancreas. "Babies who weigh under 2.5 kg at birth have poorly developed or small pancreas. Mothers should be given al the necessary supplements and nutrients to have a healthy baby that weighs between 2.5 and 3.5 kg, which is the ideal weight for an Indian baby at full term," says diabetologist V. Mohan.

Around 30 per cent of underweight newborns die within their first year. Pregnant women should be treated for anaemia, and given nutritious food, vitamin and folic acid supplements to ensure normal growth of the fetus, Dr Mohan said.

DOHaD

——“健康与疾病的发育起源”

低出生体重（LBW）

2. 宫内发育迟缓

- 胎儿窘迫
- 新生儿窒息
- 围产儿死亡

流行病学调查

宫内发育迟缓（IUGR）

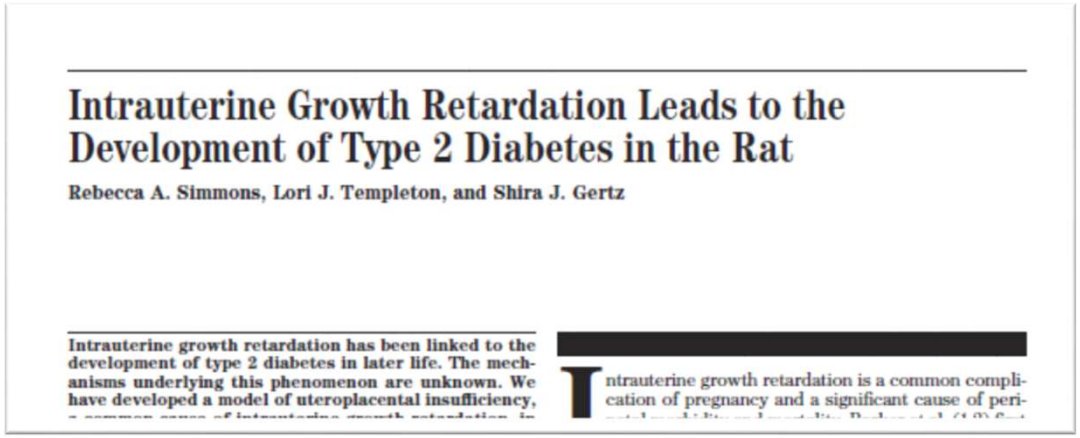
最常见的发育毒性类型
主要表现为低出生体重
多由不良宫内环境所致

出生后：
- 体格、智力发育落后
- 糖耐量异常和II型糖尿病易感

糖尿病的宫内发育起源之一

Kieran Matharu, NeoReviews 2004

3. 动物模型研究



Intrauterine Growth Retardation Leads to the Development of Type 2 Diabetes in the Rat

Rebecca A. Simmons, Lori J. Templeton, and Shira J. Gertz

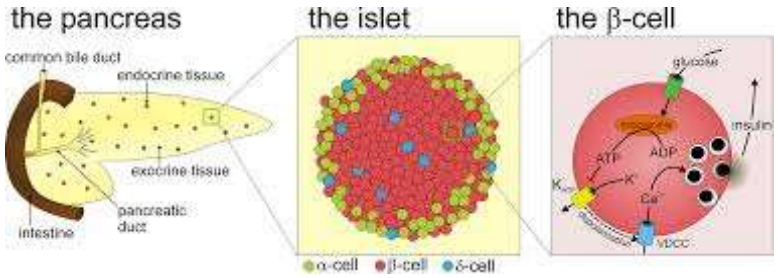
Intrauterine growth retardation has been linked to the development of type 2 diabetes in later life. The mechanisms underlying this phenomenon are unknown. We have developed a model of uteroplacental insufficiency,

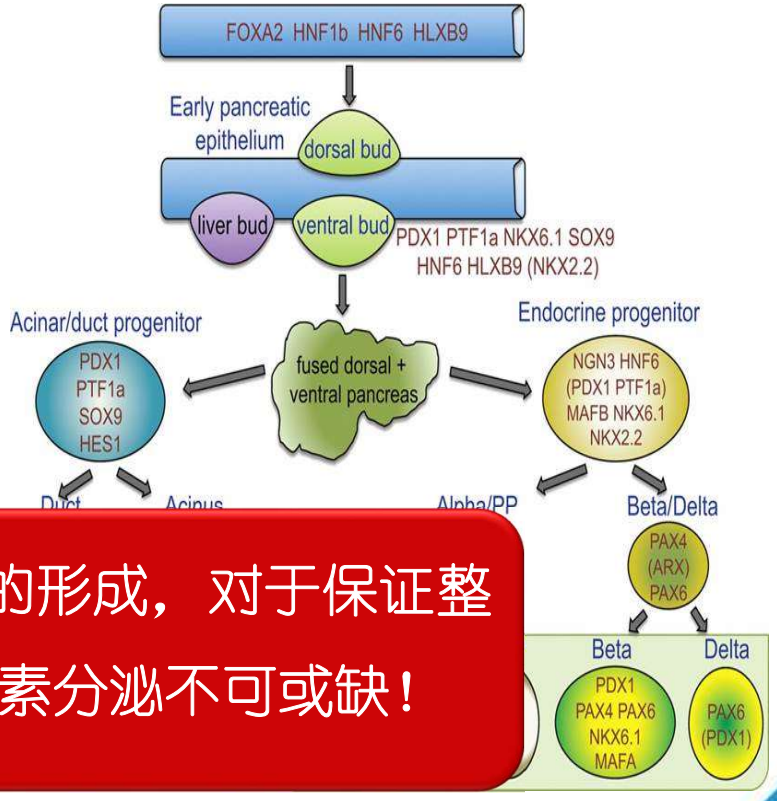
Intrauterine growth retardation is a common complication of pregnancy and a significant cause of peri-

Rebecca Simmons

- 胎盘-子宫功能不足模型(子宫动脉结扎模型)
- 孕期摄食限制模型
- 孕期蛋白限制模型
- 孕期地塞米松暴露模型

4. 胚胎胰腺发育分化



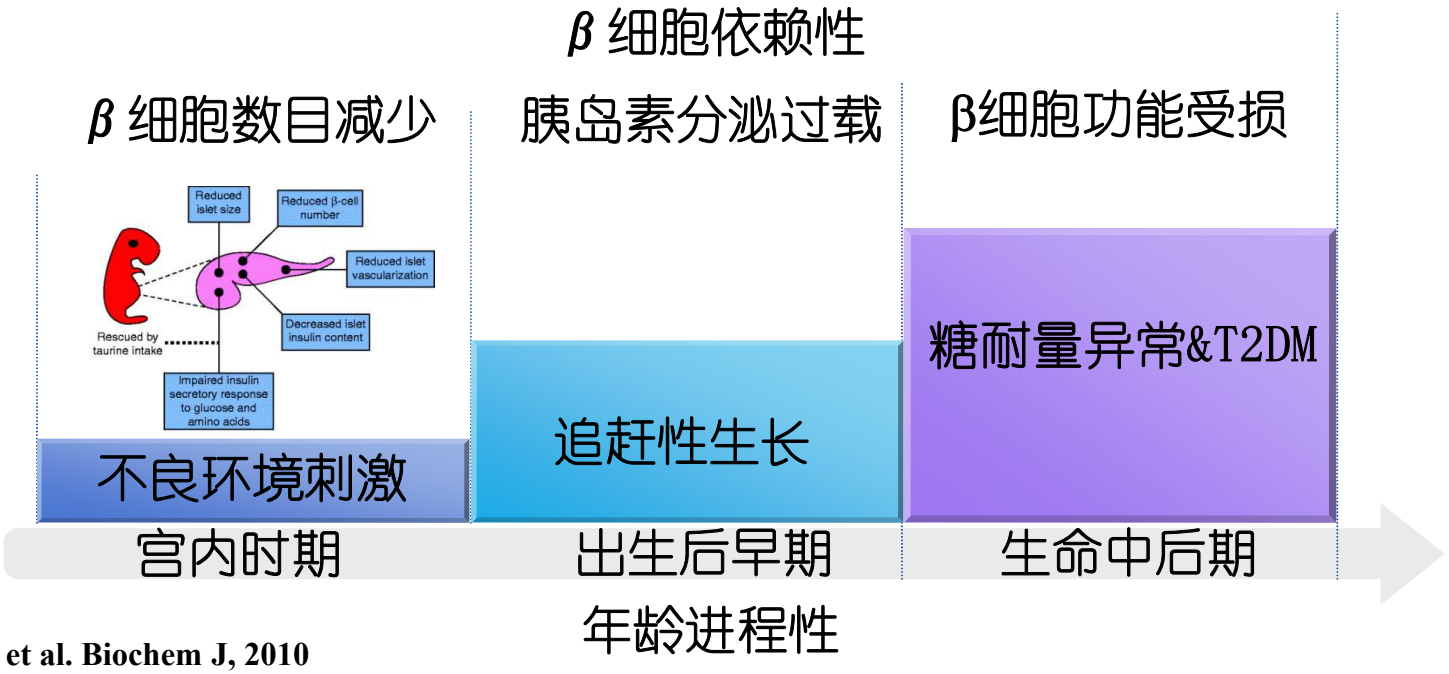


- 胚胎胰腺发育经多种转录因子按精确时-空顺序表达诱导分化形成胰岛 β 细胞
- 胰岛 β 细胞数
体细胞的定向
- 胰岛形态和功
不大

胚胎发育时期足够数目β细胞的形成，对于保证整个生命进程中正常水平的胰岛素分泌不可或缺！

Mfopou J K et al. Diabetes 2010;59:2094-2101

5. 不良宫内环境与IUGR成年T2DM易感



Warner MJ et al. Biochem J, 2010

Inoue T et al Biomed Res, 2009

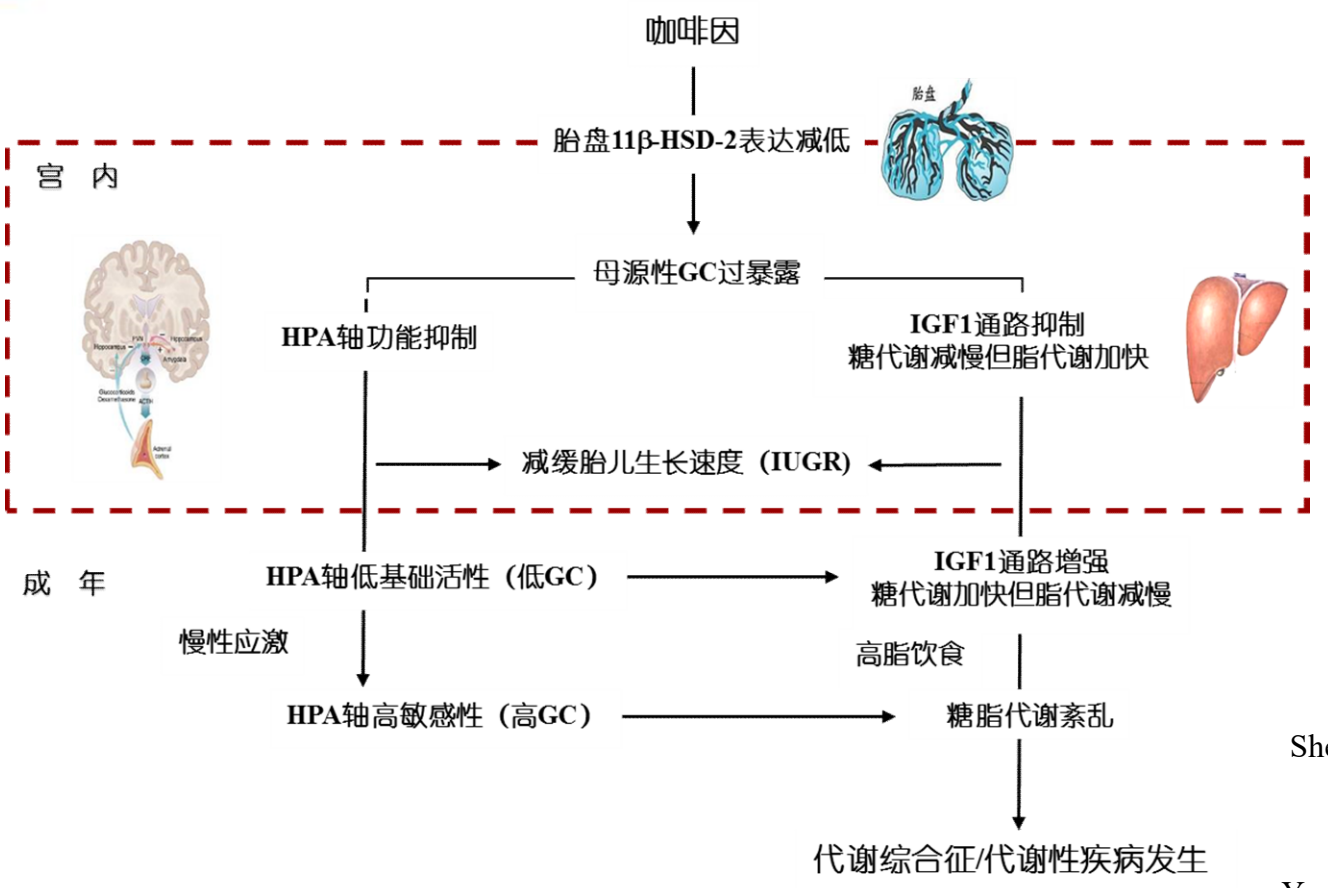
IUGR宫内发育时期β细胞数目不足和出生后“追赶性生长”驱动的胰岛素持续性高分泌共同作用所致的β细胞功能受损可能是诱发T2DM的重要原因之一

6. 咖啡因与IUGR

Bakker, R, Am J Clin Nutr, 2010
Group, C.S. BMJ, 2008
James, J., et al. BMJ, 2004

- 广泛存在于咖啡、茶、软饮料和药物中
- 具有生殖和发育毒性；能导致IUGR
- 儿童期摄入增加成年肥胖等代谢综合征风险

“神经内分泌代谢编程机制”



Xu, et al. Toxicol Appl Pharmacol 2012
Xu D, et al. Toxicol Lett 2012
Liu L, et al. Toxicol Lett 2012
Shen L, et al, Toxicol Appl Pharmacol 2014
Wang LL, et al. Toxicol Lett 2014
Ping J, et al. Toxicology 2014
Yan YE, et al, Toxicol Appl Pharmacol 2014

图1. 孕期咖啡因暴露所致子代代谢综合征及相关疾病易感的神经内分泌代谢编程机制

糖皮质激素与胰腺分化

- 宫内基础GC水平是调节胎儿组织形态和功能成熟的关键
- 发育时期，GC可以直接影响胰腺细胞定向分化，对于胰腺内外分泌腺细胞数平衡的维持有着深远的影响

临床调查

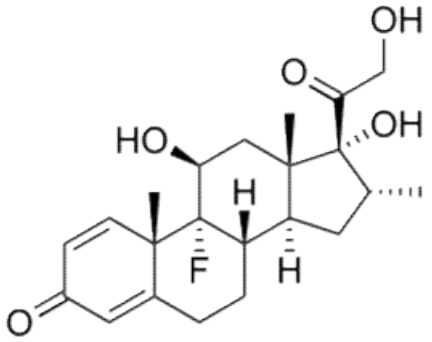
胎儿体循环GC水平与β细胞和胰岛数目负相关

动物实验

IUGR胎鼠出现母源性**GC**过暴露的同时胎鼠胰腺β细胞数目和胰岛数量均显著性降低

体外研究

GC处理体外培养大鼠胚胎胰芽可致β细胞数目减少，**Pdx-1**表达下调



糖皮质激素(GC)

Fowden, A.L *Proc Nutr Soc*, 1998.
Gesina, E., et al *Diabetes*, 2004
Blonde, B. Am J Physiol, 2001

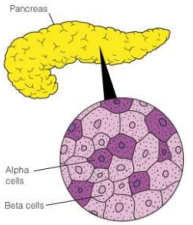
IGF-1信号通路与β细胞发育

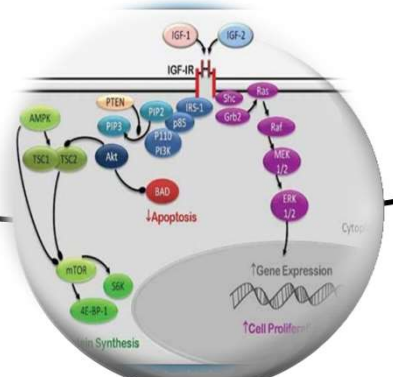
内分泌调节系统的核心

早期：肝脏合成
血流运输
晚期：自身合成
自/旁分泌

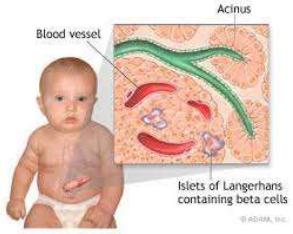
新生β细胞增殖 ↑
成熟β细胞凋亡 ↓

前体细胞扩增 ↑
间接影响
β细胞的分化





IGF-1 signaling



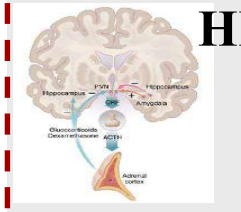
Smith FE et al. Proc Natl Acad Sci U S A, 1991
van Haeften TW et al. Eur J Clin Invest, 2004

科学问题与假说

宫 内

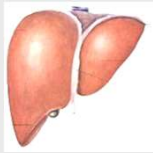
咖啡因

母源性GC过暴露



HPA轴低活性

IGF-1通路抑制

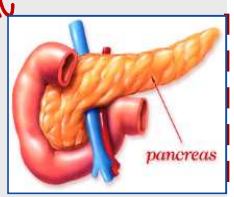


β细胞增殖、分化

胰岛素合成降低

胰腺组织形态异常

功能分化抑制



出生后

HPA轴

低基础活性（低GC）

IGF-1通路增强

（追赶性生长）

糖代谢功能改变

高脂饮食 高龄

糖耐量减低

成 年

提纲

1. 引言
2. 研究内容
3. 全文结论

研究内容

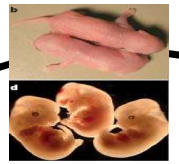
1、孕期咖啡因暴露所致胎鼠胰腺发育不良和糖代谢表型改变及其发生机制

2、孕期咖啡因暴露所致成年子代大鼠胰腺形态功能和糖代谢动态变化

Part I

孕期咖啡因暴露所致的

胎鼠胰腺发育不良和糖代谢表型改变及其发生机制



整体动物实验

动物处理与指标检测

Wistar大鼠按雌、雄(2:1)合笼

次晨检查精子/阴栓确定受孕时间

孕11~20日灌胃给予120 mg/kg 咖啡因

孕20日胎鼠 —— 体重&IUGR率

- 血咖啡因检测
  - GC/MS
- 糖代谢血表型
  - 生化/ELISA
- 胰腺形态计量和产物检测
  - HE/电镜
- 胰腺组织形态和超微结构
  - HE/电镜
- 胰腺相关基因的mRNA和蛋白表达
  - RT-PCR/免疫组化

1. 咖啡因抑制胎鼠生长发育

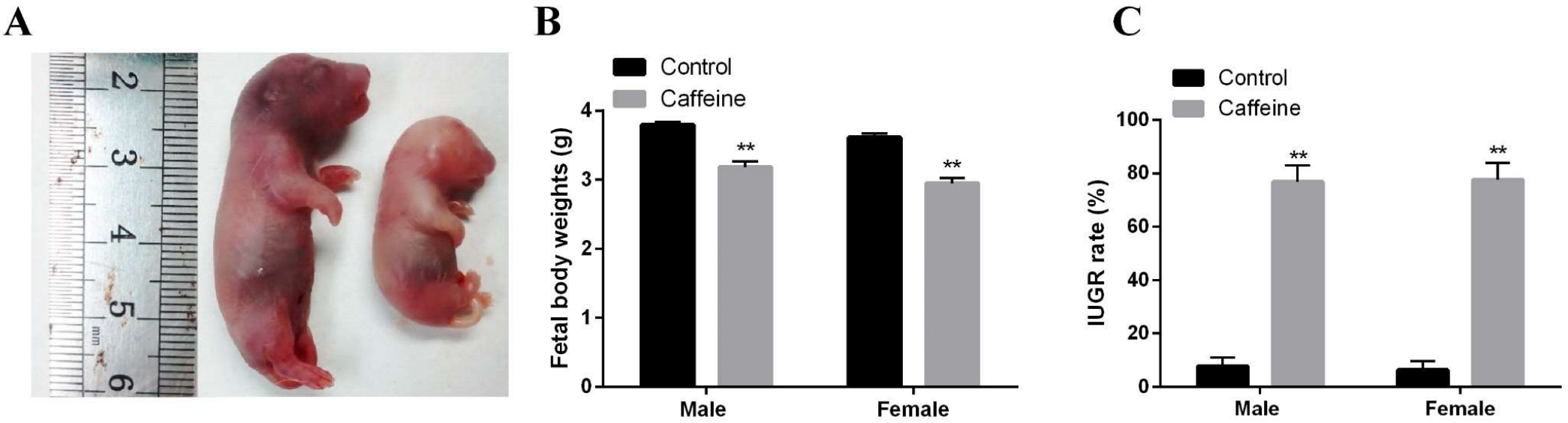


Figure 1-1 Effects of prenatal caffeine exposure on fetal growth and development in fetal rats on gestational day 20. (A) Fetal general morphology; (B) Fetal bodyweights; (C) Intrauterine growth retardation (IUGR) rates.

2. 咖啡因可通过胎盘进入胎鼠体内

血清咖啡因保留时间为**7.95 min**。母血和胎血咖啡因浓度分别为：**49±1 μg/ml**和**30±3 μg/ml**，胎血咖啡因浓度为母血的**61.2%**

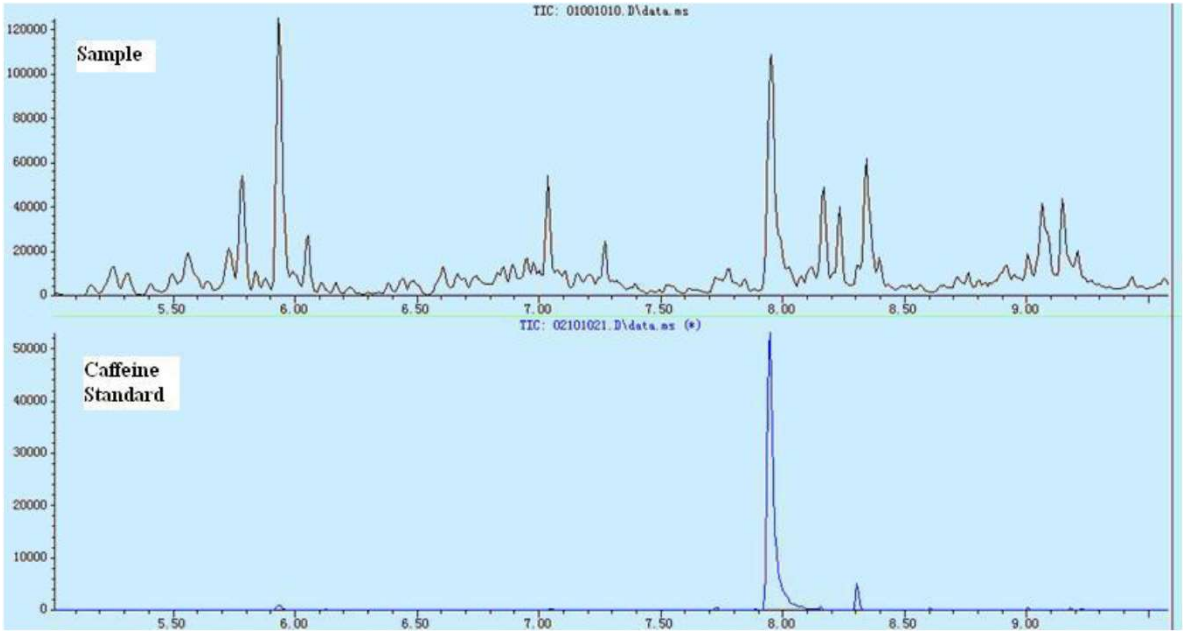


Figure 1-2 Representative gas chromatography of fetal serum caffeine content after prenatal caffeine exposure.

3. 咖啡因引起胎鼠胰岛素相关糖代谢血表型改变

待发表数据

Figure 1-3 Effects of prenatal caffeine exposure on serum glucose metabolic phenotype in maternal and fetal rats on gestational day 20.

4. 胎胰腺组织学和β细胞超微结构

待发表数据

(A)Male control; (B) Female control

(C) Male caffeine; (D) Female caffeine

“咖啡因组胰岛小，数量减少，无明显病理改变”

Figure 1-4 Effects of prenatal caffeine exposure on pancreatic morphology in fetal rats on gestational day 20.

- 分泌颗粒数目减少，囊泡内胰岛素颗粒的膜融合和脱落现象明显
- 线粒体水肿，粗面内质网扩张

待发表数据

Figure 1-5 Effects of prenatal caffeine exposure on pancreatic ultrastructure in fetal rats on gestational day 20

5. 咖啡因抑制胎鼠胰腺形态分化和胰岛素生物合成

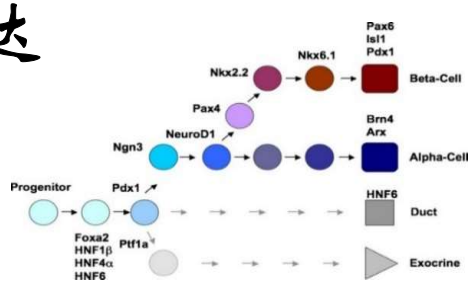
Table 1-7 Effects of prenatal caffeine exposure on pancreatic morphological development in fetal rats on gestational day 20.

待发表数据

待发表数据

Figure 1-6 Effects of prenatal caffeine exposure on pancreatic proinsulin and insulin contents in fetal rats on gestational day 20.

6. 咖啡因抑制胎胰腺发育和功能基因的表达



胰腺发育分化级联通路示意图

待发表数据

Figure 1-7 Effects of prenatal caffeine exposure on mRNA expression levels of pancreatic function-related genes in fetal rats on gestational day 20.

6. 咖啡因抑制Pdx-1和胰岛素蛋白表达

待发表数据

Figure 1-8 Effects of prenatal caffeine exposure on pancreatic duodenum homeobox 1 (Pdx-1) and insulin protein expression in fetal rats.

7. 胎胰腺GC功能相关基因mRNA表达变化

待发表数据

Figure 1-9 Effects of prenatal caffeine exposure on mRNA expression levels of pancreatic glucocorticoid function-related genes in fetal rats on gestational day 20.

8. 咖啡因抑制雌性胎鼠胰腺局部IGF-1信号通路的功能

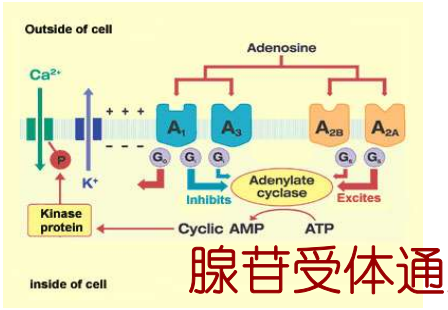
待发表数据

Figure 1-9 Effects of prenatal caffeine exposure on mRNA expression levels of pancreatic glucocorticoid function-related genes in fetal rats on gestational day 20.

9. 咖啡因改变胎胰腺苷受体的mRNA表达

待发表数据

Figure 1-11 Effects of prenatal caffeine exposure on mRNA expression levels of pancreatic adenosine receptors in fetal rats on gestational day 20.



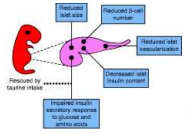
腺苷受体通路

讨论：咖啡因所致胎胰腺形态和功能变化及其分子基础

胰岛和β细胞面积与数量↓

胰腺胰岛素原和胰岛素含量↓

胰岛素分泌颗粒数目↓



胎胰腺形态发育
与功能分化异常

现象

机制

Pdx-1及下游胰腺发育级联通路↓

胰岛素功能和修饰基因↓

β细胞分化
胰岛素合成

β细胞和前体
细胞增殖

发育与功
能基因

直接抑制

经由转录因子C/EBPs

母源性GC
过暴露

GR表达↑

胎胰腺11β-HSDs表达弱，GC代谢调节能力低下

IGF-1信号通路

血IGF-1水平↓

胰腺IGF-1R、IRS1/2表达↓

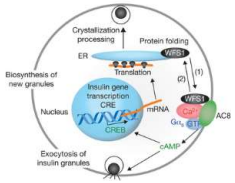
讨论：咖啡因所致IUGR胎鼠的糖代谢表型改变

母体血糖升高 ≠ 血糖降低

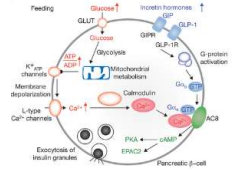
胎鼠糖代谢血表型

血胰岛素原和胰岛素升高

胰岛素合成减少（组织胰岛素原和胰岛素含量降低）

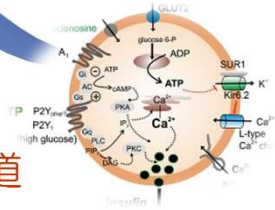


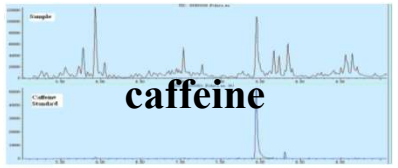
葡萄糖刺激胰岛素分泌功能抑制（Glut-2/Gck表达降低）



血胰岛素原/胰岛素比值升高

胰岛素“合成减少，释放过度”





文献报道

- 咖啡因能够改善糖尿病大鼠糖耐量，表现为胰岛素分泌增多
- 特异性Adora1受体拮抗剂能够促进大鼠体外培养的胰岛释放胰岛素

孕期咖啡因暴露导致IUGR胎鼠胰腺发育不良和糖代谢表型改变，其发生机制可能于以下两方面有关：

（1）咖啡因所致“宫内母源性GC过暴露”：不仅引起胎鼠胰腺局部CR活化，直接下调Pdx-1及其下游的胰腺发育级联信号通路和胰岛素功能基因表达，而且通过C/EBPs抑制胰腺IGF-1信号通路，间接影响β细胞及其前体的增殖，最终导致胰腺形态发育和功能分化异常。

（2）咖啡因直接作用：通过腺苷受体直接刺激胎胰腺局部的胰岛素过度释放，引起胰岛素“合成-分泌”稳态平衡失调，并导致胎鼠糖代谢表型改变。

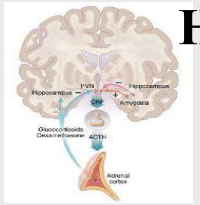
科学问题与假说

宫 内

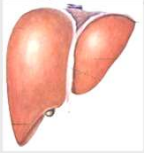
咖啡因

母源性GC过暴露

HPA轴低活性

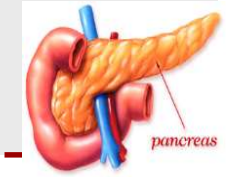


IGF-1通路抑制



β细胞增殖、分化
胰岛素合成降低

胰腺组织形态异常
功能分化抑制



出生后

HPA轴低基础活性
(低GC)

IGF-1通路增强
(追赶性生长)

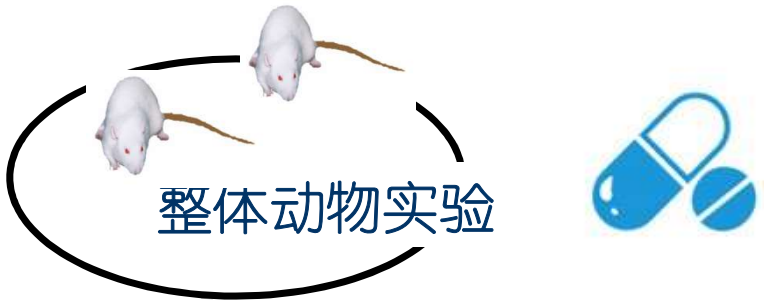
糖代谢功能改变

糖耐量减低

成 年

Part II

孕期咖啡因暴露所致成年子代大鼠胰腺形态功能和糖代谢动态变化



动物处理与指标检测

Wistar大鼠按雌、雄(2:1)合笼

次晨检查精子/阴栓确定受孕时间

孕11~20日灌胃给予120 mg/kg 咖啡因

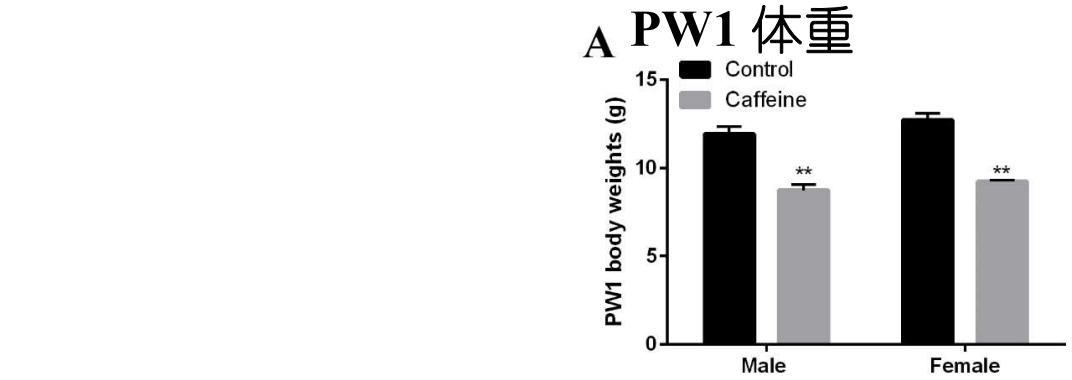
自然生产/正常饮食喂养

PW12 **PW24**

IPGTT
ITT

生长发育情况	血糖/胰岛素	β细胞形态计量	肝脏胰岛素信号通路表达
体重变化	生化/放免	免疫组化	**RT-PCR**

1. 咖啡因致IUGR子代体重出现部分“追赶性生长”



出生后：
绝对体重降低，体重增长率升高

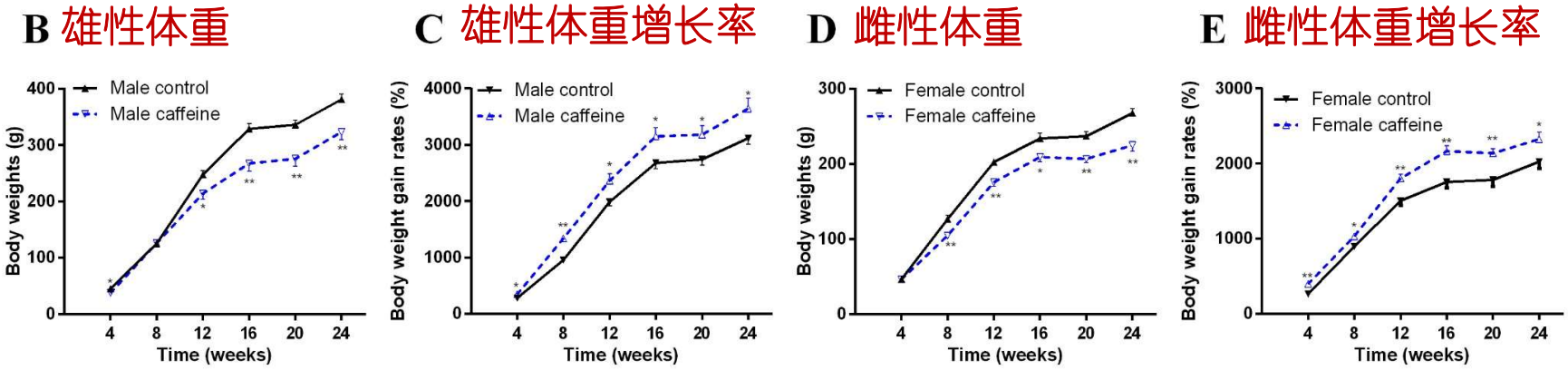


Figure 2-1 Effects of prenatal caffeine exposure on postnatal growth and development in offspring rats fed by normal diet.

2. 咖啡因至IUGR子代PW12胰岛功能不足和糖耐量减低

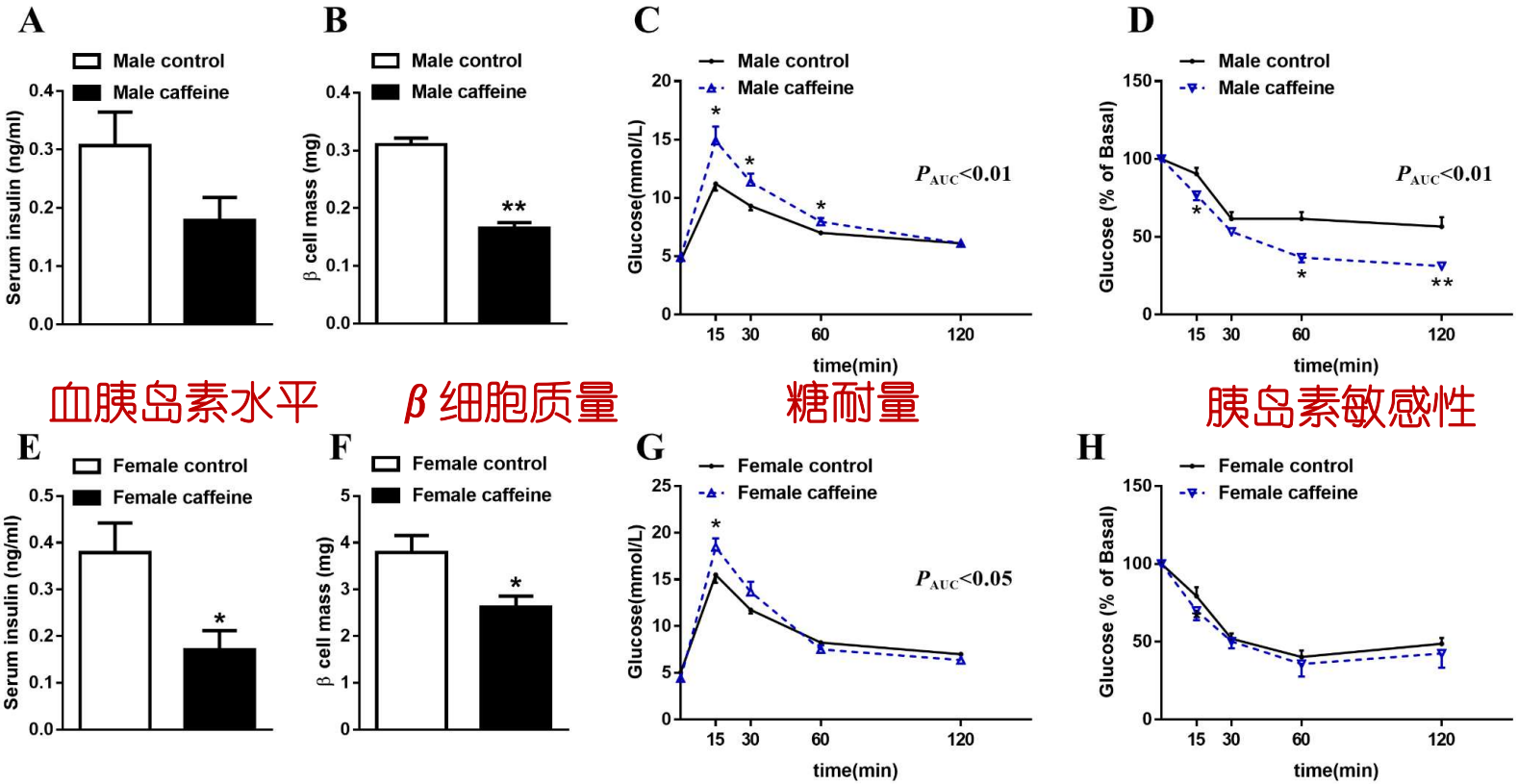


Figure 2-2 Effects of prenatal caffeine exposure on β cell mass, serum insulin and glucose tolerance in adult offspring rats at postnatal week 12.

糖耐量减低与胰岛素β细胞功能不足有关！

3. 咖啡因所致IUGR子代PW24糖耐量转强

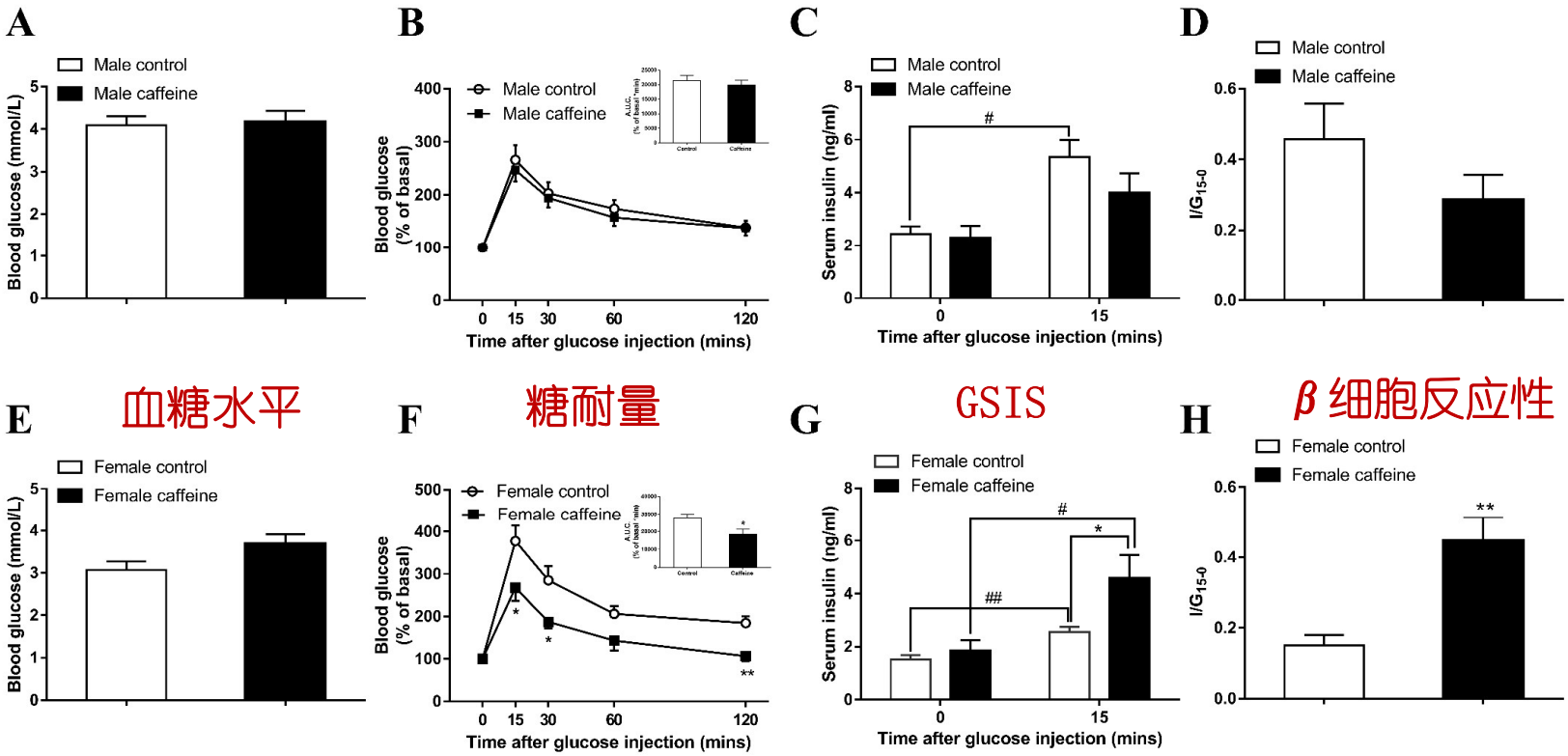
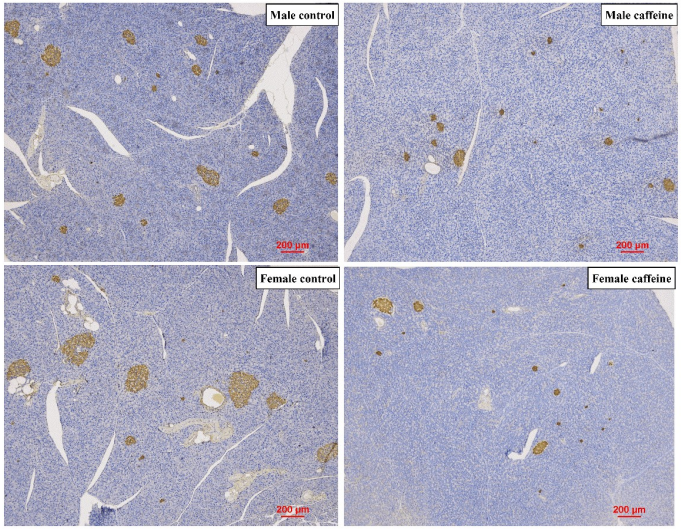
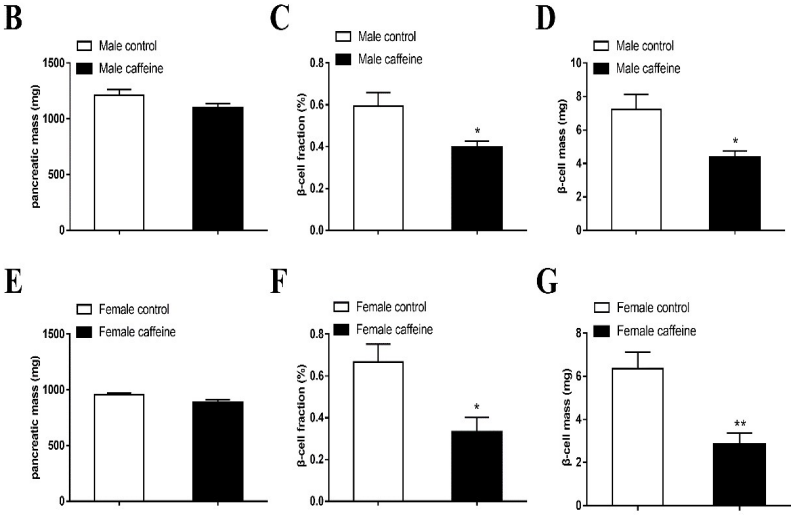


Fig. 2-3 Effects of prenatal caffeine exposure on basal glucose-insulin phenotype, glucose tolerance and β cell responsiveness to glucose in adult offspring rats.

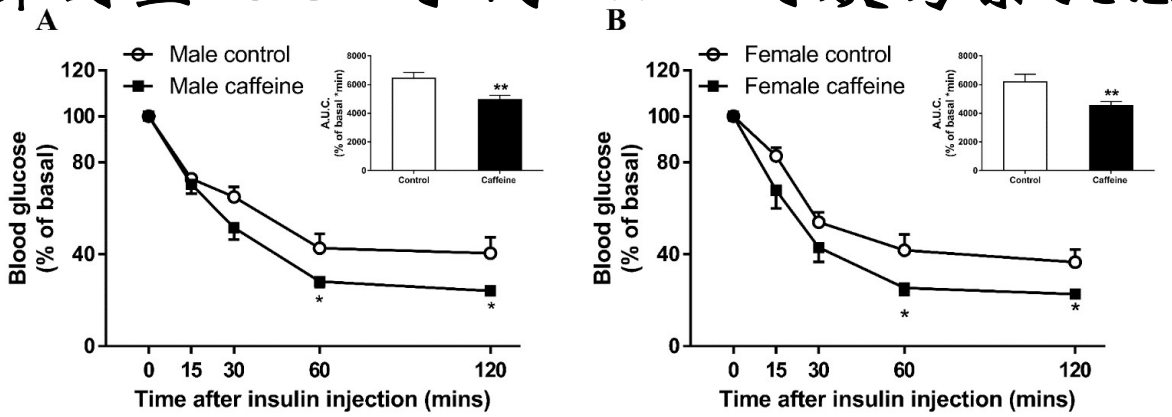
血胰岛素水平无明显改变，糖耐量增加

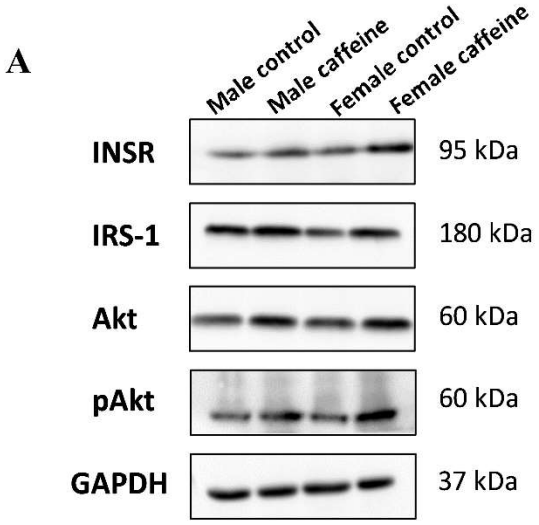
3. 咖啡因至IUGR子代PW24β细胞质量持续降低

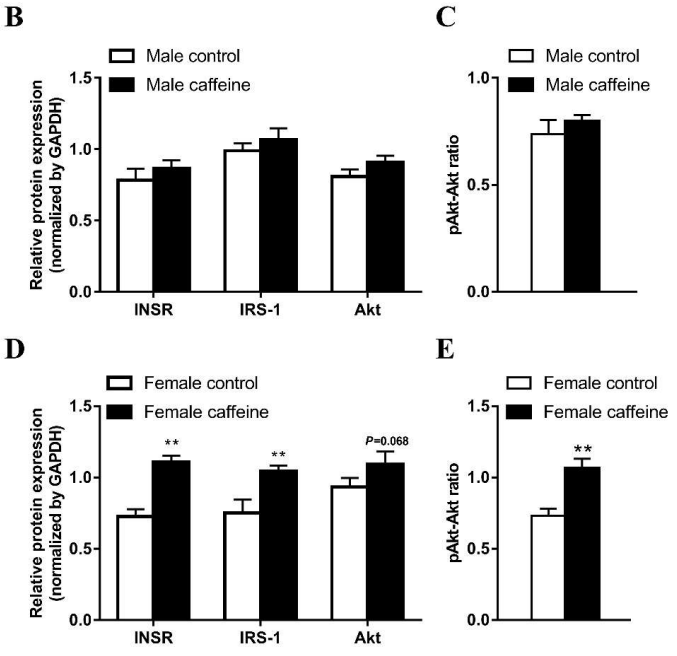




4. 咖啡因至IUGR子代PW24时胰岛素敏感性增加







机体胰岛素敏感性进一步增加与肝脏胰岛素信号通路上调有关

讨论：咖啡因所致IUGR子代成年后糖代谢功能发生改变

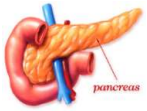
正常饮食模型

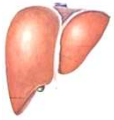
糖耐量

β细胞依赖的胰岛素分泌

胰岛素利用效率（胰岛素敏感性）

部分追赶性生长





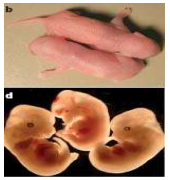
胰腺β细胞比例（数量）

肝脏胰岛素/IGF-1信号通路

宫内编程

胰腺形态发育受限

肝脏胰岛素/IGF-1信号通路抑制



提纲

1. 引言
2. 研究内容
3. 全文结论

Sci Nat (2017) 104:89
https://doi.org/10.1007/s00114-017-1510-4

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ORIGINAL PAPER

Effects of prenatal caffeine exposure on glucose homeostasis of adult offspring rats

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Received: 10 January 2017 / Revised: 30 August 2017 / Accepted: 30 September 2017

Abstract Epidemiological evidences show that prenatal caffeine exposure (PCE) could induce intrauterine growth retardation (IUGR). The IUGR offspring also present glucose intolerance and type 2 diabetes mellitus after maturity. We have previously demonstrated that PCE induced IUGR and increased susceptibility to adult metabolic syndrome in rats. This study aimed to further investigate the effects of PCE on glucose homeostasis in adult offspring rats. Pregnant rats were administered caffeine (120 mg/kg/day, intragastrically) from gestational days 11 to 20. PCE offspring presented partial catch-up growth pattern after birth, characterizing by the increased body weight gain rates. Meanwhile, PCE had no significant influences on the basal blood glucose and insulin phenotypes of adult offspring but increased the glucose tolerance, glucose-stimulated insulin section and β cell sensitivity to glucose in female progeny. The insulin sensitivity of both male and female PCE offspring were enhanced accompanied with reduced β cell fraction and mass. Western blotting results revealed that significant augmentation in protein expression of hepatic insulin signaling elements of PCE females, including insulin receptor (INSR), insulin receptor substrate 1 (IRS-1) and the phosphorylation of serine–threonine protein kinase (Akt), was also potentiated. In conclusion, we demonstrated that PCE reduced the pancreatic β mass but increased the glucose tolerance in adult offspring rats, especially for females. The adaptive compensatory enhancement of β cell responsiveness to glucose and elevated insulin sensitivity mainly mediated by upregulated hepatic insulin signaling might coordinately contribute to the increased glucose tolerance.

Keywords Prenatal caffeine exposure · Intrauterine growth retardation · Glucose tolerance · Insulin sensitivity · Pancreatic β cell development · Hepatic insulin signaling

Introduction

Intrauterine growth retardation (IUGR) is the failure of a fetus to achieve a predicted growth potential based on the genetic constitution and environmental influences, and it primarily manifests as low birth weight. Approximately 5 to 10% of newborns worldwide are characterized by IUGR (Resnik 2002), and the incidence of IUGR in some developing countries reaches as high as 30% (Saleem et al. 2011). Epidemiological studies have shown that detrimental intrauterine environment (i.e., maternal malnutrition, protein deprivation) insults pancreatic development and permanently "programs" β cell mass and function in IUGR offspring, and these irreversible alterations may greatly increase the risk of developing glucose intolerance and type 2 diabetes mellitus (T2DM) in late period of life (Bo et al. 2000; Yajnik 2000; Kahn 2001). IUGR animal models established by different methods also displayed diabetic symptoms in adult offspring (Garofano et al. 1997; Bertin et al. 1999; Garg et al. 2013).

Communicated by: Sven Thatje

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Published online: 09 October 2017

Springer

结 论

- 孕期咖啡因暴露所致IUGR成年子代胰腺β细胞数量不足可一直延续到出生后，导致成年后早期胰岛素总体合成分泌不足，引起糖耐量减低。
- 追赶性生长时期，胰岛素/IGF-1信号通路持续性高表达可能通过增加单位胰岛素合成分泌并不断提高机体对胰岛素的敏感性，从而代偿β细胞数量不足对循环胰岛素水平的影响，最终改善甚至逆转糖不耐受。
- 孕期咖啡因暴露所致IUGR成年子代胰腺β细胞数量不足和肝脏胰岛素/IGF-1信号通路高表达存在宫内编程效应。

Thank You