

高脂饮食对于肠道基因表现的影响

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摘 要

本研究透过次世代定序与大数据的分析, 研究高脂饮食对仓鼠肠道组织基因表现。将16只仓鼠分成对照组和高脂组。在第12周将高脂组改摄取控制组饲料, 并于第4、8、12和20周时, 每组牺牲2只仓鼠进行分析。研究结果发现, 摄取4、8、12周高脂饲料后, 基因表现变化量达2倍以上的基因有51~328个, 再以Ingenuity Pathway Analysis (IPA)生物路径分析相关基因表现后, 发现有许多关于细胞凋亡、过氧化反应、发炎反应与细胞增生等相关基因表现的生物路径相较于控制组有较高程度的诱发。在第20周, 高脂组仍然可以发现脂质生成作用的相关基因表现与DNA甲基化及转录抑制讯息传导基因。同时, 肠道上皮细胞亦启动了多项关于伤害修复的调控机制, 例如: glutathione-mediated detoxification, nucleotide excision repair。本实验证实, 在停止高脂喂食后, 基因表现与长期高脂喂食的非常类似, 但同时伴随修补基因进行损伤修复。

关键词

高脂饮食, 基因表现, DNA修复

Effect of High Fat Diet on Intestinal Gene Expression in Hamster

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Abstract

Next-generation sequencing and big data analysis were used to investigate the high-fat diet on gene expression level of intestinal tissues in hamster. Sixteen hamsters were assigned to the control group and the high-fat group, respectively. At week 12, the high-fat group was switched from high-fat to the control diet. At weeks 4, 8, 12, and 20, two hamsters from each group were sacrificed for analysis. The results showed that 51~328 genes exhibited more than a twofold change in expression level in high-fat diet group at week 4, 8, 12. Subsequently, Ingenuity Pathway Analysis (IPA) was used to analyze the relative gene expression levels. We found that significantly increased expression levels of genes associated with cell apoptosis, oxidative stress, inflammation, and cell proliferation compared to the control group. At week 20, the high-fat group still exhibited gene expressions related to lipid biosynthesis, DNA methylation, and transcriptional repression signaling, but concurrently, several regulatory mechanisms for damage repair were activated in intestinal epithelial cells, including Glutathione-mediated detoxification and nucleotide excision repair. This study demonstrated that the gene expressions at stopping the intake of high fat diet were similar to those of long-term high-fat feeding, but it was accompanied with upregulation of repair genes.

Keywords

High Fat Diet, Gene Expression, DNA Repair

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1. 引言

由于社会的快速变迁,生活步调与型态日渐改变,连锁餐饮业不断增加,国人外食饮食习惯的养成,造就了现代常见的文明病“高血脂症”。在过去的研究中发现高脂饮食与糖尿病、高血压、中风与心脏病等皆有相关[1][2],更有前人在小鼠的研究中发现高脂饮食会引发肠道中 sulfidogenic bacteria 硫化菌的大量繁殖,进而引起肠道中发炎反应相关之 monocyte chemoattractant protein 1 (Mcp1), Tolllike receptor 4 (Tlr4)等基因的大量表现,且上皮组织中有屏蔽功能的紧密结合蛋白(Zonula Occludens-1, ZO-1)受到损害进而引起发炎反应[3]。在过去的研究中也发现,肠道总基因组(metagenome)的组成与体内新陈代谢身体免疫等功能息息相关[4][5][6][7][8],肠道微生物组成的改变与第二型糖尿病(Type II DM)有关[4],更有研究发现肠道总基因组的组成可能透过遗传的方式来影响下一代,而且某些肠道菌属(如: Christensenellaceae)的大量繁殖与人体的代谢功能有关[8],因此,肠道微生物的组成将会影响人体的代谢、疾病和营养状况,而前人的研究亦指出不同的饮食习惯下可能导致肠道菌种的组成有所改变[9]。在高脂喂食诱导小鼠产生肥胖和胰岛素抗性的研究中,发现大量小肠基因表现的变化,特别是在有关脂质代谢的调控[10][11][12]。小鼠研究中发现会高脂饮食喂食会造成小肠的发炎反应与肥胖[13]。因此本研究利用次世代定序平台提供定序资料并透过大数据分析高脂饮食对肠道上皮组织转录子与基因代谢路径表现差异。

2. 材料与方法

2.1. 实验动物饲养

自财团法人实验研究院实验动物中心购买 8 周龄雄性仓鼠(golden Syrian hamster)共 16 只, 随机分为 2 组, 分别为控制组(corn starch 45.2%, sucrose 20%, casein 20%, soybean oil 5%, AIN-93M mineral 3.5%, AIN-93M vitamin 1%, methylated cellulose 5%, DL-methionine 0.3%)和高脂组(corn starch 28%, sucrose 20%, casein 20%, soybean oil 5%, lard 17%, AIN-93M mineral 3.5%, AIN-93M vitamin 1%, methylated cellulose 5%, DL-methionine 0.3%, cholesterol 0.2%)。除了 sucrose 和 soybean oil 购自卖场, 其他饲料成分购自 MP Biomedicals (Solo, OH, USC)。高油脂组动物摄取高脂饲料至 12 周后改摄取对照组饲料至第 20 周。二组仓鼠分别于第 4 周、8 周、12 周及 20 周牺牲。牺牲时以吸入性麻醉气体二氧化碳(CO₂)麻醉, 以眼窝采血方式收集血液, 收集小肠(十二指肠、空肠、回肠)后, 将小肠以无菌生理食盐水将外部窗洗干净后, 转移至 15 毫升离心管, 并加入 5 毫升 RNeasy Lysis Buffer (Qiagen)溶液, 先放置于 4 度冰箱 18 小时后, 转移至 -80 度冰箱保存。由于肠道组织抽取 RNA 后发现有个别样本抽出之品质经鉴定后 RNA Integrity Number (RIN)数值小于 5.0, 因此删除品质不佳样本, 改以每个组别 2 个样本进行定序与基因表现分析。本实验经实践大学实验动物照护及使用委员会审核通过(编号: 10514)。

2.2. 总核糖核酸萃取与次世代定序

利用 Tri 试剂萃取总核糖核酸(Total RNA; Ambion, Austin, TX, USA)后, 使用 NanoDrop ND-1000 分光光度计(Thermo Scientific)与 Agilent 2100 生物分析仪(Agilent Technologies, Palo Alto, CA)来确认 RNA 浓度和品质。最佳的 RNA 品质应介于 A260/A280 = 1.8~2.0, RNA 完整度, RIN > 8.0, 随后将 RNA 浓度调整为 200 ng/ul。所萃取出的总量 1~2 微克 RNA 再利用含 Poly-T 之磁珠纯化出 mRNA, 纯化完后依循 Illumina 公司的定序试剂组(Stranded mRNA Library Prep.)所制定的程序, 进一步制备 cDNA 基因库(cDNA Library), 首先利用二价阳离子借由 95 度的高温将 RNA 进行片段化, 每个 RNA 片段大小约为 160~200 个核苷酸, 随后以反转录酶与随机引子合成 cDNA, 第二步骤于 cDNA 之 5'与 3'两端接上定序用之引子, 完成后进行聚合酶链锁反应, 将已接上定序引子的 cDNA 放大成为最终之 cDNA 基因库。最末定序部分使用 NovaSeq6000 定序平台(Illumina Inc.), 以双尾定序法定序 cDNA 基因库两端各 150 个核苷酸。

2.3. 定序资料分析

使用 Fastqc 进行定序品质确认后, 以 MesAur1.0 (Ensembl genome browser 91)为参考基因组, 将 reads 回贴至此参考基因组, 并进行基因注解与定量。定量的计算方式采用 TPM (Transcripts Per Kilobase Million)进行标准化。差异表现分析以 $abs(\log_2FC)$ 大于等于 2 或 4 来筛选出比较组别间有显著差异的基因。

功能性注解的部分, 由于此物种较新, 常用的注解工具像是 GO/KEGG, 尚无提供此物种的支援, 所以将差异表现的基因序列提取出来后, 与 human/mouse/rat 进行 blast 后, 找出相对应的基因名后, 进行 Ingenuity Pathway Analysis (IPA)分析。

3. 结果

3.1. 高脂饲料对于仓鼠体重、食物利用效率的影响

除了第 4 周的高油脂组体重的增加显著高于控制组, 在第 8、12、20 周二组皆无显著差异。实验期间控制组饲料摄取量显著高于高油脂组, 但是各组的食物利用效率除了第四周的高油脂组显著高于控制组, 第 8、12、20 周二组间皆没有统计上的显著差异(data not shown)。

3.2. 血清胆固醇浓度

控制组第 4、8、12 周之间血清总胆固醇浓度没有统计上的显著差异，第 20 周显著下降。高脂组第 4、8 周和第 8、12 周之间没有统计上的显著差异，第 20 周则是显著低于第 12 周。比较各周控制组和高油脂组，第 4 周的高油脂组和控制组没有统计上的显著差异，在第 8、12、20 周的高油脂组皆显著高于控制组(data not shown)。

3.3. 转录体表现差异分析

原始数据过滤(paired-end raw reads): 利用 Trimmomatic 软体分别对双端的原始数据(raw reads)进行质量过滤(V0.33, <http://www.usadellab.org/cms/?page=trimmomatic>), 过滤含未知(N)的 reads、质量值低于 20 及过滤后序列长度低于 100 bp 的 reads, 得到通过质量控制后的 paired-end clean reads (表 1、表 2)。

Table 1. Quality control of next-generation sequencing data of control group

表 1. 控制组次世代定序质量

group	C 4-1 ¹	C 4-2	C8-1	C 8-2	C 12-1	C 12-1	C 20-1	C 20-2
QC Assessment								
Q20	99.38	99.32	98.32	99.43	99.55	99.58	98.95	98.52
Q30	89.51	89.11	87.59	88.27	91	90.98	88.45	86.83
GC	51.5	51.5	48	44	47.5	47.5	41.5	49.5
Alignment								
Total input reads	8,143,779	7,666,869	5,168,767	1,243,689	6,537,159	3,997,637	3,926,078	3,416,860
Average input read length	152	152	152	152	152	152	152	152
Unique Reads								
Uniquely mapped reads	7,355,633	6,962,959	460,894	587,890	3,901,494	3,282,054	3,259,942	987,401
% of uniquely mapped reads	90.32%	90.82%	8.92%	47.27%	59.68%	82.10%	83.03%	28.90%
Chimeric Reads								
Number of chimeric reads	66,757	76,750	2608	5456	49,003	42,488	27,970	8691
% of chimeric reads	0.82%	1.00%	0.05%	0.44%	0.75%	1.06%	0.71%	0.25%

¹C 为控制组，4、8、12、20 为周数，1、2 为编号。

Table 2. Quality control of next-generation sequencing data of high-fat group

表 2. 高油脂组次世代定序质量

group	H4-1 ¹	H4-2	H8-1	H8-2	H12-1	H12-2	H20-1	H20-2
QC Assessment								
Q20	99.06	99.25	99.15	98.95	98.7	99.07	99.17	99.16
Q30	85.26	89.28	89.03	88.27	88.29	88.06	85.73	87.29
GC	47	51	48.5	44	48	49	48.5	48.5

Continued								
Alignment								
Total input reads	2,891,051	2,677,499	377,801	381,698	1,187,221	5,284,996	3,670,092	3,403,667
Average input read length	152	152	152	152	152	152	152	152
Unique Reads								
Uniquely mapped reads	2,194,156	2,235,142	61,802	3,285,081	501,686	4,718,648	2,989,881	3,004,345
% of uniquely mapped reads	75.89%	83.48%	16.36%	84.10%	42.26%	89.28%	81.47%	88.27%
Chimeric Reads								
Number of chimeric reads	16,678	23,366	519	610	3740	53,483	29,914	32,798
% of chimeric reads	0.58%	0.87%	0.14%	0.16%	0.32%	1.01%	0.82%	0.96%

¹H 为高油脂组；4、8、12、20 为周数；1、2 为编号。

以 MesAur1.0 (Ensembl genome browser 91)为参考基因组，将 reads 回贴至此参考基因组，并进行基因注解与定量。定量的计算方式采用 TPM (Transcripts Per Kilobase Million)进行标准化。差异表现分析是以 $\text{abs}(\log_2\text{FC})$ 大于等于 2 或 4 来筛选出比较组别间有显著差异的基因(表 3)。研究结果发现，喂食高脂饮食 4~12 周后，肠道组织的基因表现与控制组比较发现，基因表现差异达两倍以上基因数随着喂食周数增多而增加，而更改喂食一般饲料至 20 周的组别，高脂喂食组与控制组基因表现差异达 4 倍以上的基因亦显著增加。

Table 3. Analysis of changes in intestinal gene expression
表 3. 肠道基因表现分析

HF/ CON	H4/C4 ¹	H8/C8	H12/C12	H20/C20
$\text{abs}(\log_2\text{FC})$	≥ 2	≥ 2	≥ 2	≥ 4
DEGs	150	354	618	963
Blast to Mammals	120	331	621	883
IPA mapped genes	51	172	328	469

¹C 为控制组；H 为高油脂组，4、8、12、20 为周数。

IPA 生物路径分析已广泛地运用于系统生物学与讯息路径分析，我们将各组所找到的具表现差异之基因，以 IPA 进行分析后，可以建构出相关文献中目标基因功能与其他分子间的调控关系，并挑选出排名前五项目代表性的生物路径调控关系(表 4)。

4. 讨论

在本研究中，喂食 4 至 12 周高脂饲料的仓鼠经基因表现分析与 IPA 分析后，我们在高油脂喂食组发现肠道组织上有许多关于细胞凋亡、过氧化反应、发炎反应与细胞增生等相关基因表现的生物路径相较于控制组有较高程度的诱发。

Table 4. Ingenuity Pathway Analysis (IPA) (top 5 canonical pathways for each group)
表 4. IPA 生物路径分析(前 5 项最显著调控路径)

	Ingenuity Pathway Analysis (top 5)	$-\log(p\text{-value})^2$
H4/C4¹	Lipid Antigen Presentation by CD1	2.81
	Nur77 Signaling in T Lymphocytes	2.13
	Calcium-induced T Lymphocyte Apoptosis	2.02
	PXR/RXR Activation	1.98
	Pyruvate Fermentation to Lactate	1.91
H8/C8	ILK Signaling	6.04
	Tight Junction Signaling	3.82
	Agranulocyte Adhesion and Diapedesis	3.66
	Airway Pathology in Chronic Obstructive Pulmonary Disease	2.89
	Hepatic Fibrosis/Hepatic Stellate Cell Activation	2.77
H12/C12	ILK Signaling	3.16
	Adipogenesis pathway	2.89
	PAK Signaling	2.81
	TNFR1 Signaling	2.54
	Calcium Signaling	2.48
H20/C20	Glutathione-mediated Detoxification	4.35
	Adipogenesis pathway	4.06
	DNA Methylation and Transcriptional Repression Signaling	3.6
	Glycine Degradation (Creatine Biosynthesis)	3.54
	Nucleotide Excision Repair Pathway	3.53

¹C 为控制组; H 为高油脂组, 4、8、12、20 为周数; ²The most statistically significant canonical pathways are listed according to their p value ($-\log$)。

研究以 CD1 基因剔除小鼠研究高脂喂食后, 发现较高的脂肪组织发炎程度与胰岛素抗性[14]。同时 Nur77 蛋白调控 T 细胞受体亦容易造成细胞凋亡。过去的研究发现钙离子诱发之 T 淋巴细胞产生凋亡, 进而造成 MEF2 等转录子表现上升, 增加低密度脂蛋白受体(LDLR)的表现与 LDL 的吸收, 而增加动脉粥样硬化的结果[15]。另一项小鼠研究中发现, 在 Pregnane X Receptor (PXR)基因剔除小鼠模式中, 以高脂饲料喂食后发现, PXR 受激活表现后, 会促使胆固醇合成, 并于血浆中发现丝氨酸蛋白酶(PCXK9)的表现量增加, PCXK9 会与位在肝脏细胞表面的低密度脂蛋白受体(LDLR)结合后降解, 而 LDLR 受体数目减少则会导致血清 LDL 胆固醇(LDL-C)增加[16]。另一方面, 前人研究中也发现, 血浆中胆固醇浓度过高会导致肠道组织无法获得足够的氧、或无法快速处理氧气的情况下, 丙酮酸脱氢酶(Pyruvate dehy-drogenase complex)便无法及时将丙酮酸转换为乙酰辅酶 A (Acetyl-CoA)进入有氧呼吸, 组织也因此无法借有氧呼吸获得充足能量造成丙酮酸堆积[17]。

喂食高脂饮食的小鼠一周后发现, integrin-linked kinase (ILK)表现量上升进而造成胰岛素阻抗增加与伴随发炎反应[18] [19] [20]。Tight junction signaling 则与细胞增生有关, Tight junction 蛋白可以维持肠道上皮细胞的完整性, 任何变化都容易造成肠道上皮细胞的破坏(例如: 肠道炎) [21]。Agranulocyte Adhesion

and Diapedesi 则亦与发炎反应息息相关[22], 同时与发炎反应相关的还有与慢性阻塞性肺炎(Airway Pathology in Chronic Obstructive Pulmonary Disease)有关的基因亦大量表现[23]。一项高胆固醇喂食的小鼠实验中发现, 由于游离胆固醇的增加, 易于造血干细胞中堆积, 致使 TLR4 讯息传导路径表现量改变, 而 TLR4 的增加已被证实会引发炎症反应, 从而造成肝脏纤维化[24]。前人一项以猪为模式的高脂喂食研究显示, 除体重增加外, 并发现脂质生成作用(adipogenesis)及发炎反应的基因, 如: SREBP-1c, FASN1, DGAT2 等表现量有上升的现象[25]。PAK 讯息传导中的基因表现异常则被认为与癌症的形成相关[26]。TNFR1 基因剔除小鼠的研究中发现, 缺乏 TNFR1 可以抑制饮食所引起的肥胖, 因此 TNFR1 的表现量增加则易造成肥胖的情况[27]。在我们的结果中亦发现钙离子信息传导路径的异常表现, 过去的研究发现高脂饮食喂养小鼠会抑制肝细胞中儿茶酚胺并刺激的钙离子信号传导, 最后导致脂肪肝等疾病的发生[28]。

经过 12 周的高脂喂食并恢复为一般喂食的仓鼠至 20 周后, 仍然可以发现肠道组织脂质生成作用相关的基因表现与 DNA 甲基化及转录抑制讯息传导基因表现仍迥异于控制组, 表示高脂喂食诱发之肠道脂肪生成与细胞发炎作用的现象仍旧存在[25] [29], 但是值得注意的发现是, 此时肠道上皮细胞亦启动了多项关于伤害修复的调控机制, 例如: glutathione-mediated detoxification, 在前人小鼠实验的研究中发现短期(2 周)高脂喂养的小鼠肌肉中, 麸胱甘肽(GSH)表现量降低 25%, 发炎指标白血球介素-6 (IL-6)有 4.5 倍的大量表现[30], 虽然在我们的实验中, 4 至 12 周高脂喂食组的仓鼠肠道组织与控制组相比并无显著差异, 但是恢复一般喂食至 20 周时, 却发现 GSH 的表现量显著的增加, 这样的表现可以用来抑制高脂喂食所产生的过氧化与发炎反应[31]。另外, 在此组别的仓鼠肠道组织中亦发现 nucleotide excision repair (NER) 修补机制启动进行因长时间高脂喂食对肠道组织诱发的氧化压力与损伤后的修补机制[32]。此时伴随而来的 Glycine 代谢相关基因也观察到大量表现, 并参与防止组织损伤、增加抗氧化耐受性与产生抗发炎物质等[33]。

本实验证实了取消高脂喂食一段时间后, 肠道组织虽仍存在着发炎反应的基因表现机制, 但是同时伴随大量修补基因表现的机制进行损伤修复。综合本次的肠道组织基因表现分析与之前研究肠道总体菌相分析结果[34]非常相似, 而肠道细胞所产生的这些反应是否源于细菌菌相改变而细菌产生的代谢作用的影响所导致, 是值得后续更进一步的研究与探讨。

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