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Ganoderma lucidum reduces obesity in mice by modulating the composition of the gut microbiota

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主要内容

1 背景

2 方法

3 结果

4 讨论

1 背景

1. Traditional Chinese Medicine

药用蘑菇

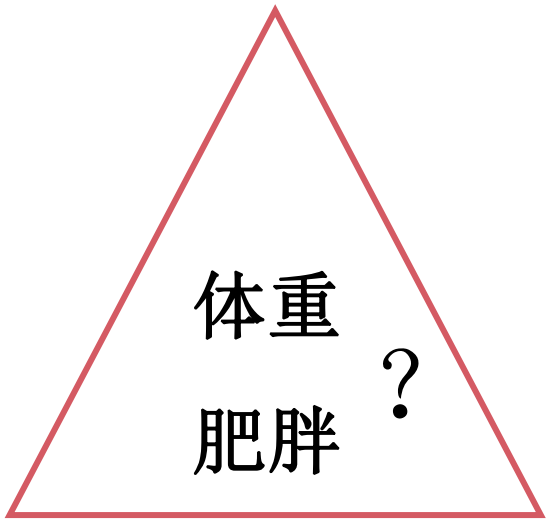
冬虫夏草、牛樟芝、姬松茸等

- 免疫调节物质
- 生物活性物质

灵芝 *Ganoderma lucidum*

- 多糖类
- 三萜类
- 蛋白聚糖类

抗糖尿病
抗高血脂
抗氧化



1 背景

2. 肥胖的危害：

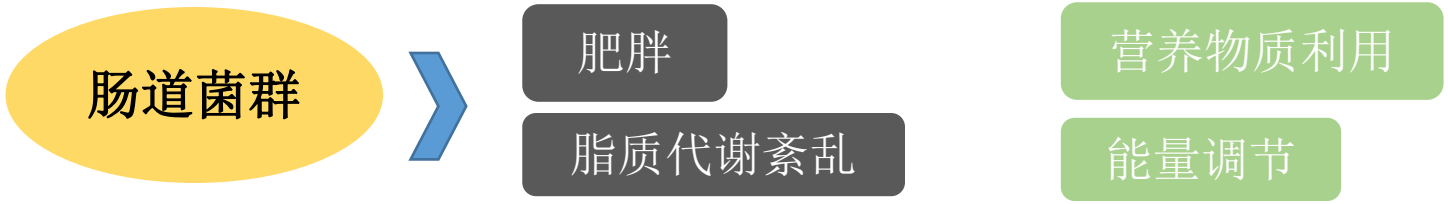
- 慢性低水平炎症
- 胰岛素抵抗
- Ⅱ型糖尿病
- 脂肪肝
- 睡眠窒息症
- 癌症、恶性肿瘤

5亿“肥胖患者”

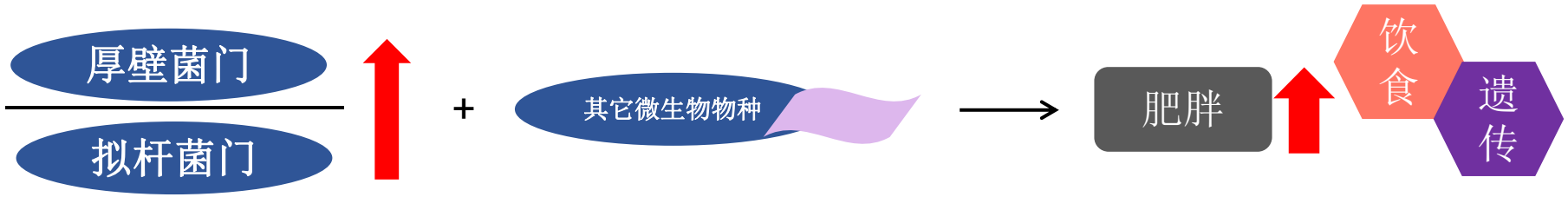
14亿“超重个体”

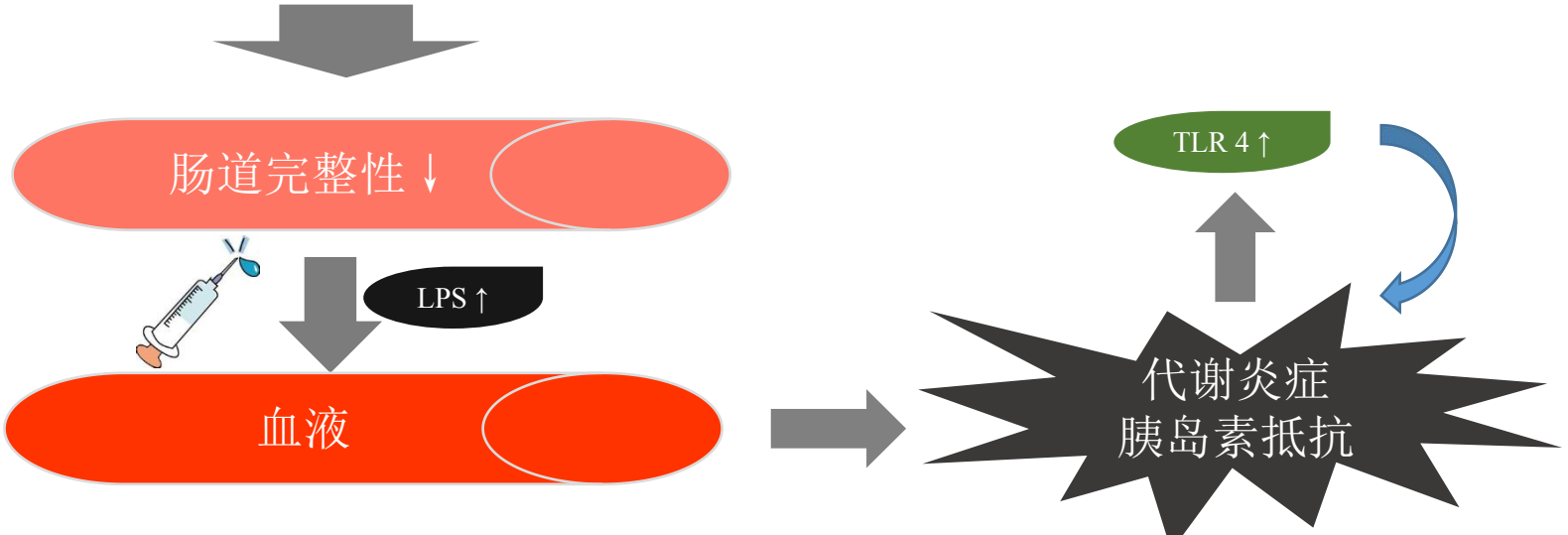
1 背景

3.


肠道菌群
肥胖
脂质代谢紊乱
营养物质利用
能量调节

4.


厚壁菌门
拟杆菌门
+
其它微生物物种
肥胖
饮食
遗传


肠道完整性↓
LPS↑
血液
TLR 4↑
代谢炎症
胰岛素抵抗

1 背景

5. 肥胖和代谢紊乱的治疗方法有哪些？

抗生素 + 益生元

改变肠道菌群

减轻内毒血症

提高葡萄糖耐受

改变肠道菌群

提高肠道的紧密连接性和完整性 → LPS ↓

瘦素缺乏小鼠/高脂饮食小鼠

（Cani, P. D. *et al*., 2009）

2 方法

1、材料

3个试验

试验动物：8周大雄性小鼠

养殖时间：3个月

分组情况：每组 5-7 只小鼠，每个笼子3-4只

饲料：

基础饲料（13.5% of energy from fat）

高脂饲料（60% of energy from fat）

水和灵芝水提物通过灌胃法添加（100μl/天）；

灵芝水体物：

粗纤维	< 5%
水解多糖	> 10%
粗蛋白	38.78%
粗脂肪	2.41%
碳水化合物	41.99%
氨基酸	5.2%
钠	76.39mg/100g
热量	345kcal/100g

2 方法

Chow
Chow+8%
HFD
HFD+2%
HFD+4%
HFD+8%

2、灵芝水提物中多糖种类分析

Chow→ HFD
8% WEGL (Chow)→ HFD
HFD→ HFD
8% WEGL (HFD)→ HFD

3、粪菌移植

（1）正式试验开始 4周后，每天收集粪便样品（无菌操作）；

（2）同一处理组粪便样品混合，每100mg粪便加入1ml无菌水重悬，剧烈涡旋10s；

（3）800g离心3min，收集上清；

移植材料在当天的移植试验前10min准备好，保证样品新鲜，微生物组成基本无变化。

2 方法

4、抗体

一抗

IkB- α

JNK (c-Jun N-terminal)

phosphorylated JNK

phosphorylated IRS-1

IRS-1 (insulin receptor substrate-1)

Akt

phosphorylated Akt

TLR4

ZO-1 (zonula occludens-1)

occludin

二抗

anti-rabbit IgG

内参

β **-actin**

anti-mouse IgG

其它

FITC-conjugated anti-F4/80

PE-conjugated anti-CD11b

anti-CD11c

PE-conjugated anti-CD4

PerCp-Cy5.5-conjugated anti-CD25

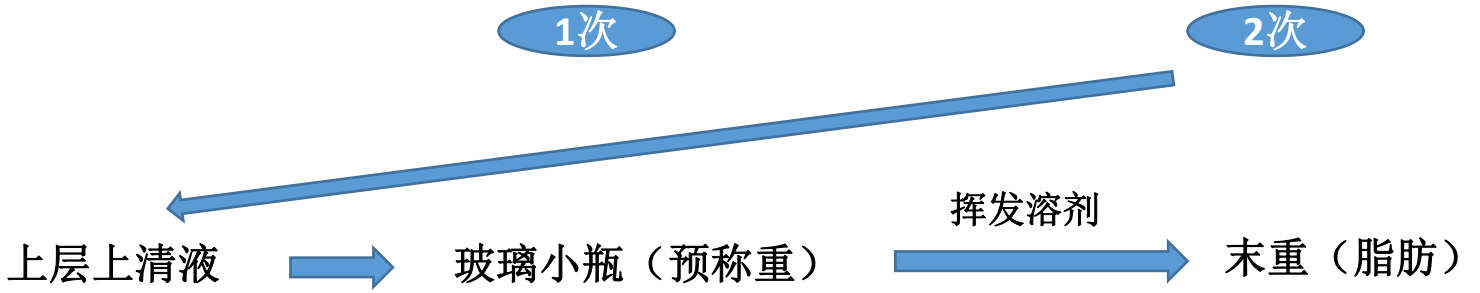
Alexa Flour 488-conjugated anti-Foxp3

2 方法

5、粪便中能量和脂肪含量测定

能量：adiabatic bomb calorimeter (Gallenkamp, UK).

脂肪含量：干燥粪便样品、庚烷/乙醚/乙醇（1:1:1, vol/vol）、庚烷/乙醚/乙醇/水（1:1:1:1, vol/vol）



6、油红O染色

肝脏冷冻切片（6μm） → 油红O染色20min → 苏木精复染1min → 光学显微镜观察

每个试验动物制作20份组织切片

2 方法

7、灌胃葡萄糖耐受检测

禁食一夜，给小鼠灌胃葡萄糖溶液（3g/kg，66%）；

尾静脉取血，使用葡萄糖测定仪测定血糖含量。

8、生物化学分析

血清LPS定量

血清胰岛素浓度测定

血清游离脂肪酸（FFA）定量

2 方法

9、qRT-PCR

10、Western blotting

脂肪组织　肝脏组织　肠道组织

11、附睾脂肪组织形态测定分析

附睾脂肪组织 → 10%福尔马林过夜处理 → 脱水 → 浸蜡 → 切片（8μm） → H.E染色 → 细胞尺寸分析（Image J software）

Supplementary Table 1. Primers used in this study

Name	Sequence
TNF-α Forward	5'-TAGCCAGGAGGGAGAACAGA-3'
TNF-α Reverse	5'-TTTTCTGGAGGGAGATGTGG-3'
IL-6 Forward	5'-CCGGAGAGGAGACTTCAC-3'
IL-6 Reverse	5'-TCCACGATTTCCCAGAGA-3'
IL-1β Forward	5'-TTGAAGAAGAGCCCATCCTC -3'
IL-1β Reverse	5'-CAGCTCATATGGGTCCGAC -3'
IL-10 Forward	5'-GCTCTTACTGACTGGCATGAG -3'
IL-10 Reverse	5'-CGCAGCTCTAGGAGCATGTG-3'
MCP-1 Forward	5'-TCACTGAAGCCAGCTCTCTCT -3'
MCP-1 Reverse	5'-GTGGGGCGTTAACTGCAT-3'
PAI-1 Forward	5'-TCAGCCCTTGCTTGCCTCAT-3'
PAI-1 Reverse	5'-GCATAGCCAGCACCGAGGA-3'
FAS Forward	5'-GCTGCGGAAACTTCAGGAAAT-3'
FAS Reverse	5'-AGAGACGTGTCACTCCTGGACTT-3'
SREBP-1c Forward	5'-GATGTGCGAACTGGACACAG-3'
SREBP-1c Reverse	5'-CATAGGGGGCGTCAAACAG-3'
ACC-1 Forward	5'-GAGTGACTGCCGAAACATCTCTG-3'
ACC-1 Reverse	5'- GCAAGGAGGACAGAGTTTATCGTG-3'
PPAR-γ Forward	5'-GCAGCTACTGCATGTGATCAAGA-3'
PPAR-γ Reverse	5'-GTCAGCGGGTGGGACTTTC-3'
ZO-1 Forward	5'-ACCCGAAACTGATGCTGTGGATAG-3'
ZO-1 Reverse	5'-AAATGGCCGGGCAGAACTTGTGTA-3'
Occludin Forward	5'-ATGTCCGGCCGATGCTCTC-3'
Occludin Reverse	5'-TTTGGCTGCTCTTGGGTCTGTAT-3'
GAPDH Forward	5'-GCATCCACTGGTGCTGCC -3'
GAPDH Reverse	5'-TCATCATACTTGGCAGGTTTC-3'
Total bacteria Forward	5'-ACTCCTACGGGAGGCAGCAG-3'
Total bacteria Reverse	5'-ATTACCGCGGCTGCTGG-3'
Firmicutes Forward	5'-GGAGYATGTGGTTTAATTCGA-3'
Firmicutes Reverse	5'-AGCTGACGACAACCATGCAC-3'
Bacteroidetes Forward	5'-GGARCATGTGGTTTAATTCGATGAT-3'
Bacteroidetes Reverse	5'-AGCTGACGACAACCATGCAG-3'

2 方法

12、流式细胞分析（Flow cytometry analysis）

脂肪组织　　肝脏

流式细胞仪：FACSCalibur (Becton Dickinson, USA)

数据分析软件：Kaluza flow cytometry analysis software (Beckman Coulter, USA).

巨噬细胞（Macrophage）　**F4/80** and **CD11c** double-positive cells

T细胞（T-cell）　**CD4**, **CD25** and transcription factor **Foxp3**

13、炎症细胞因子测定

IL-1b, IL-6 和 TNF-α 蛋白水平测定　　ELISA kits (R&D Systems, USA).

2 方法

14、肠道菌群分析

基因组DNA提取： Faecal DNA isolation kit (MoBio Laboratories, USA)

16S rRNA gene　V3–V5 regions

15、qRT-PCR定量分析特殊细菌的丰度

Firmicutes

Bacteroidetes

比值

Total bacteria

（Livak, K. J. & Schmittgen, T. D., 2001）

16、统计学分析

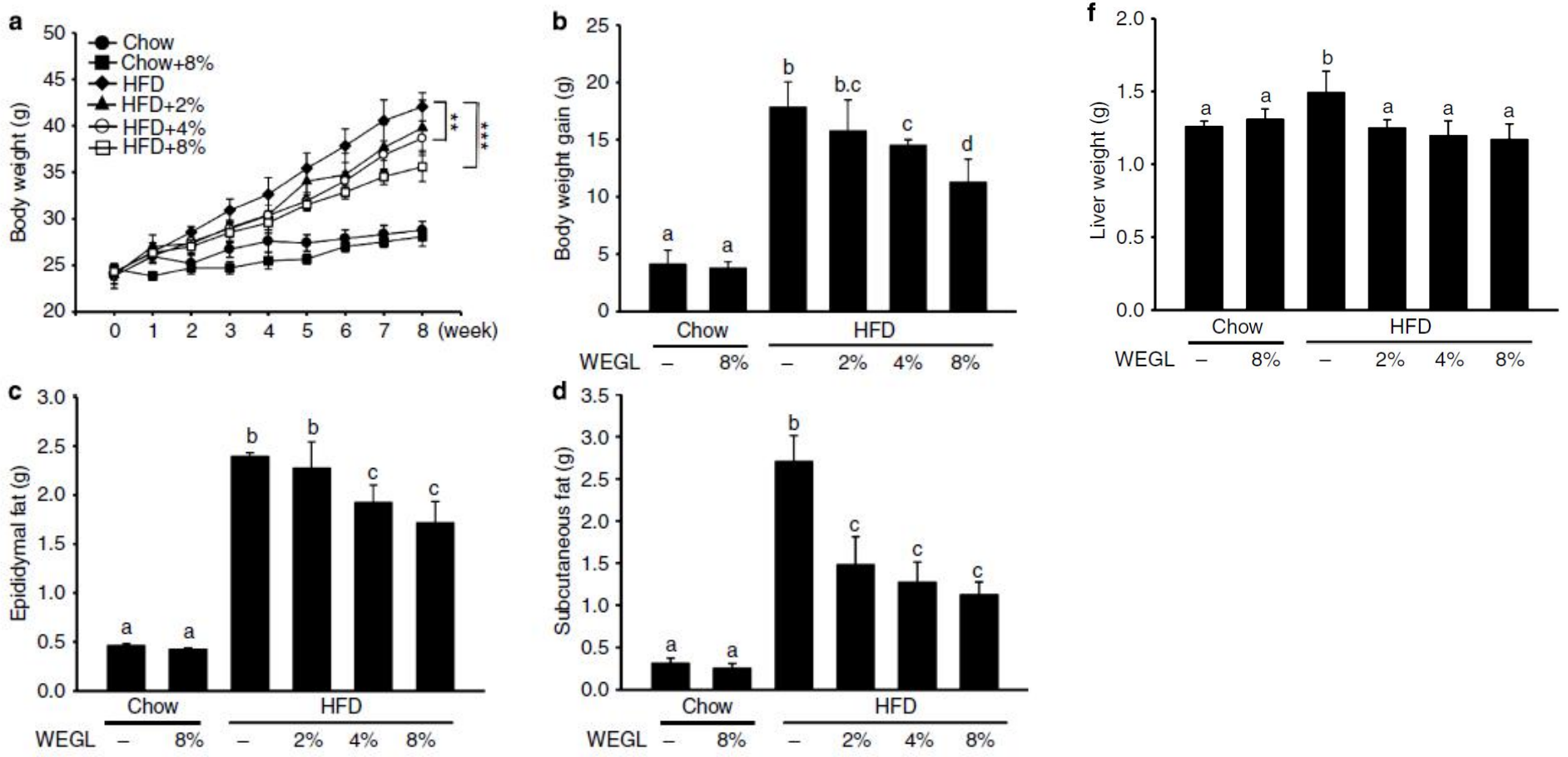
3个重复

means±s.e.m　t-test　one-way ANOVA

SPSS version 17.0

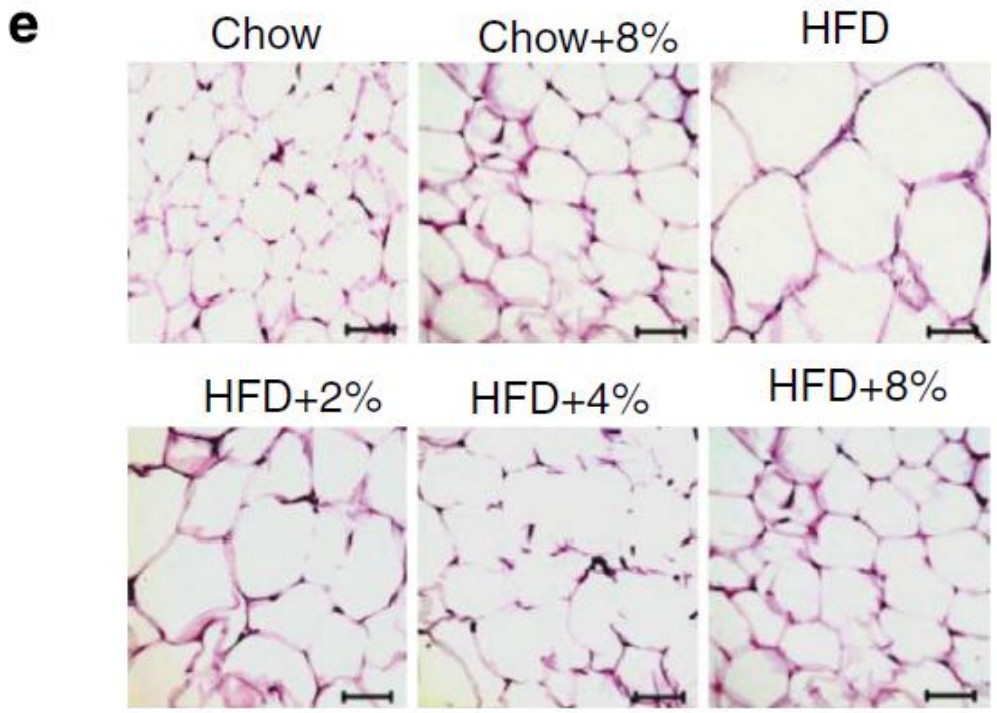
3 结果

1. WEGL prevents HFD-induced obesity in mice

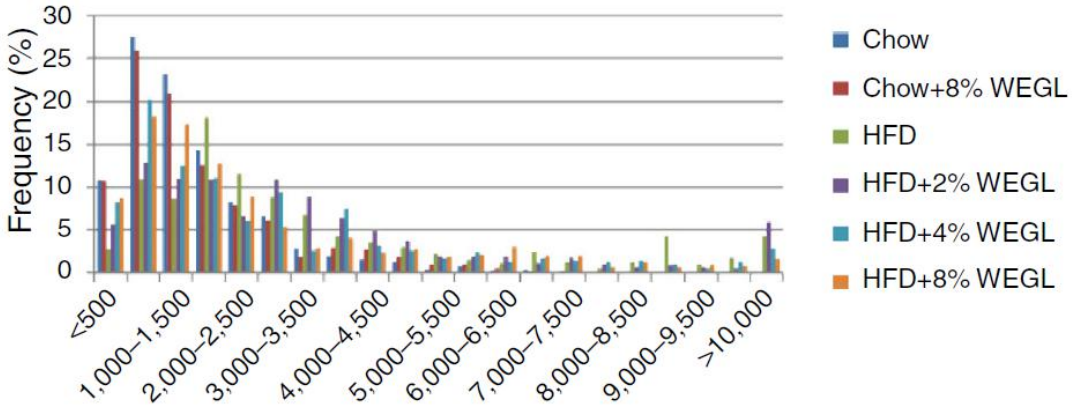


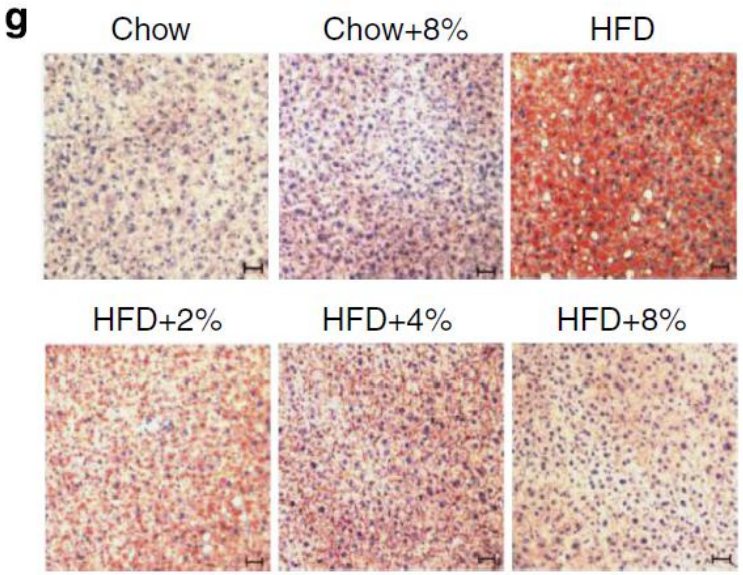
3 结果

1. WEGL prevents HFD-induced obesity in mice



附睾脂肪组织

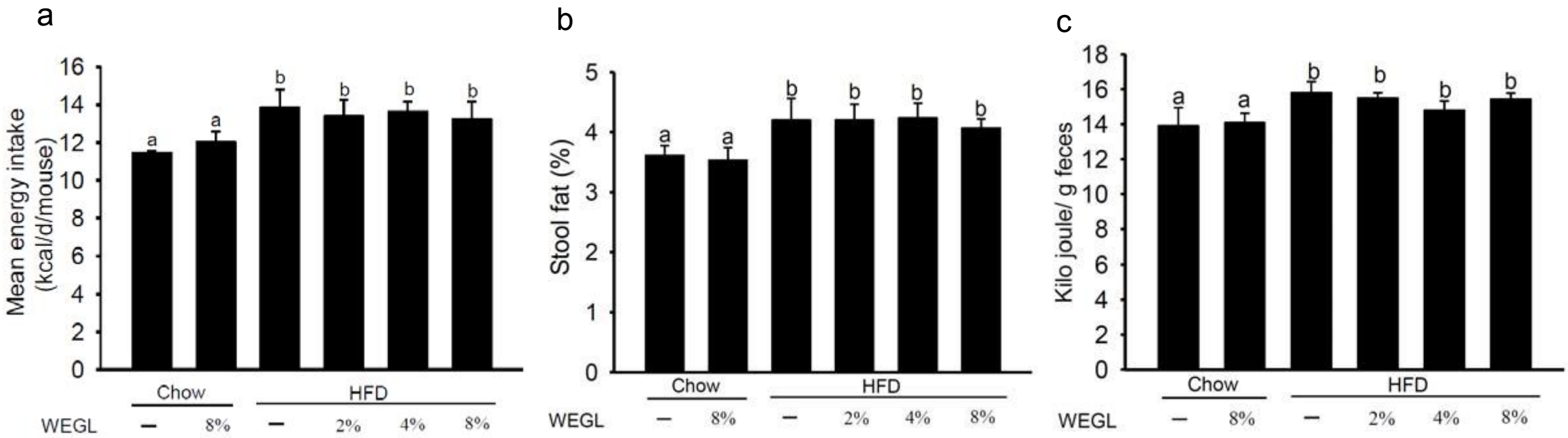




肝脏

3 结果

1. WEGL prevents HFD-induced obesity in mice

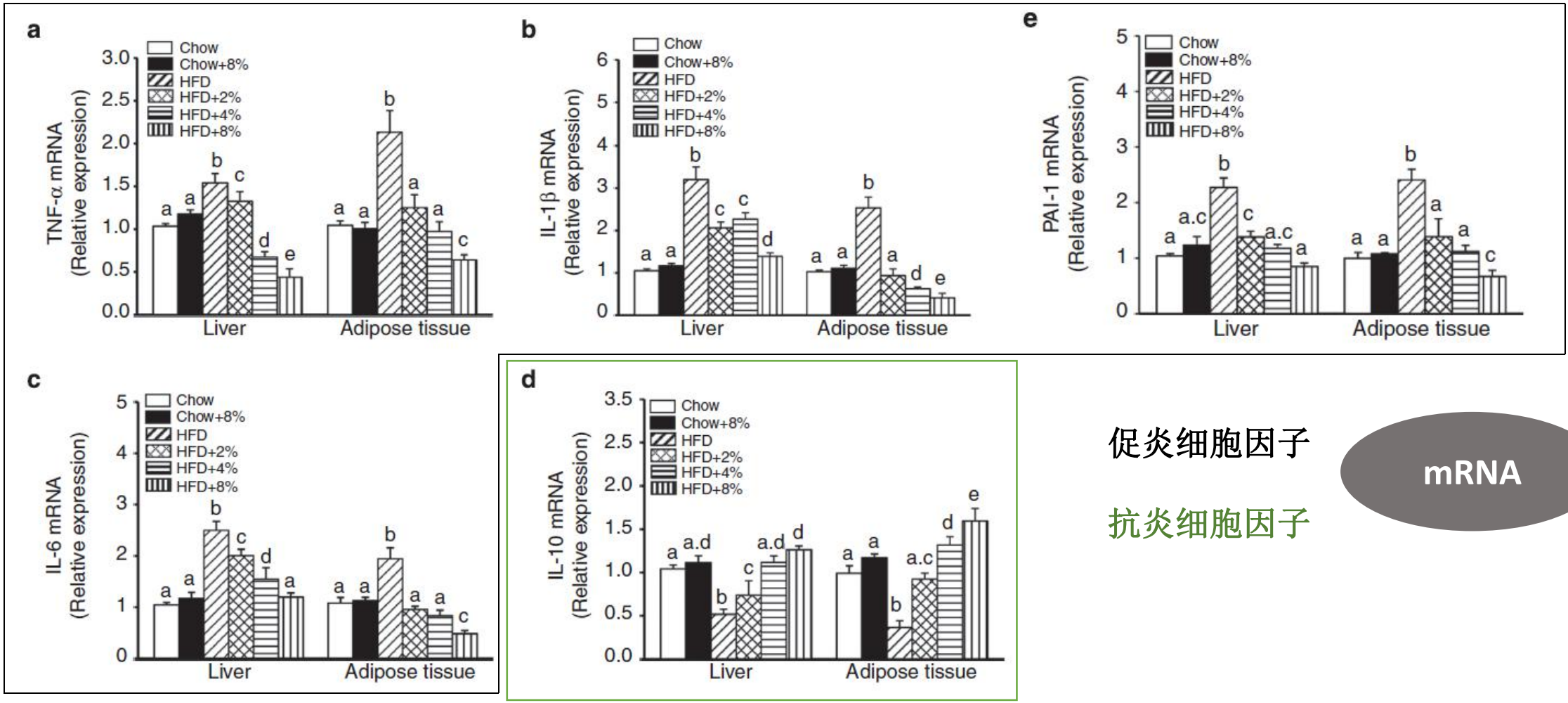


Energy intake (a), stool fat (b) and energy in feces (c) ▲ **WEGL** ~~摄食量~~ ~~能量~~

3 结果

2. WEGL reduces inflammation in HFD-fed mice

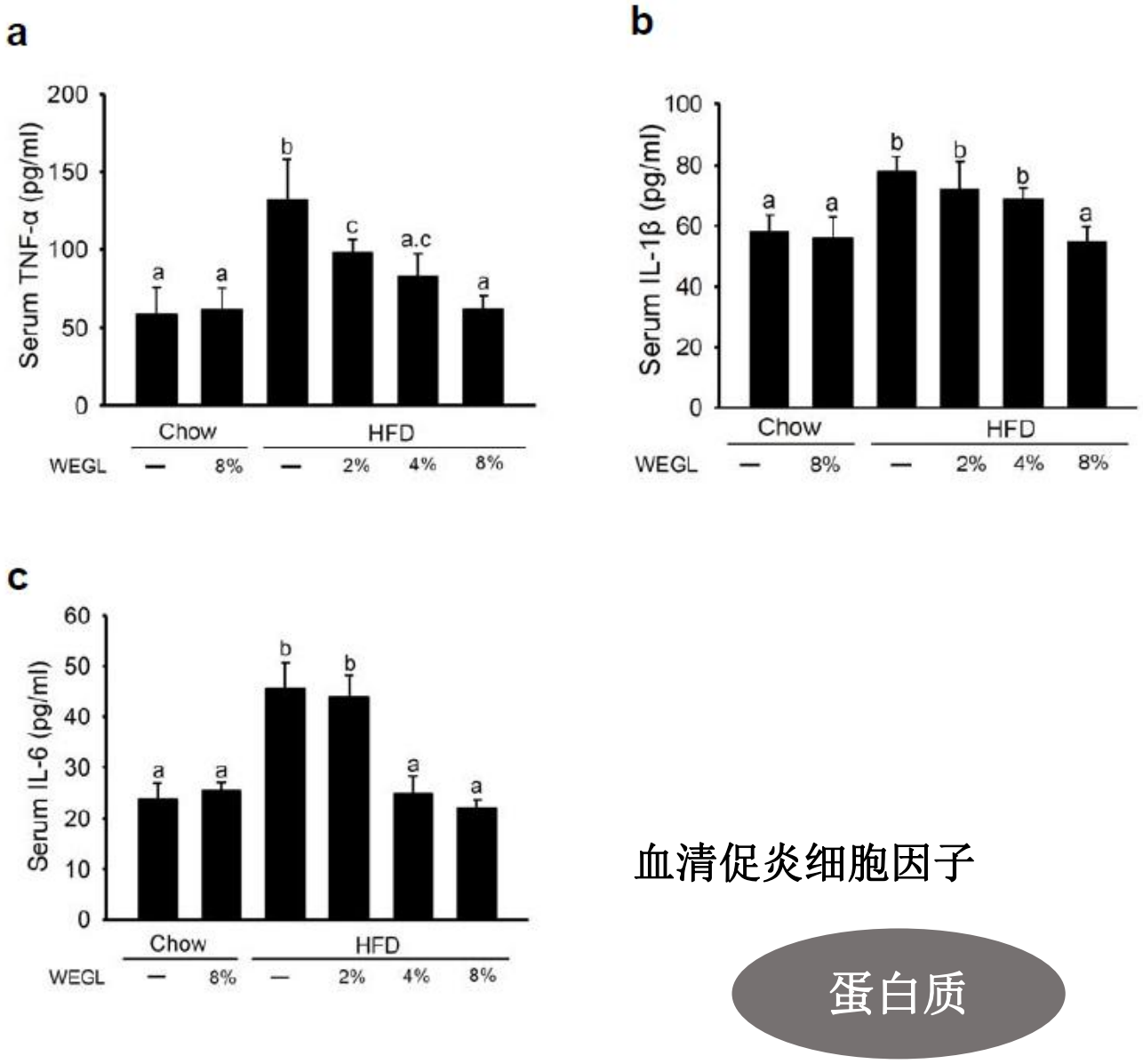
纤溶酶原激活抑制物-1



促炎细胞因子

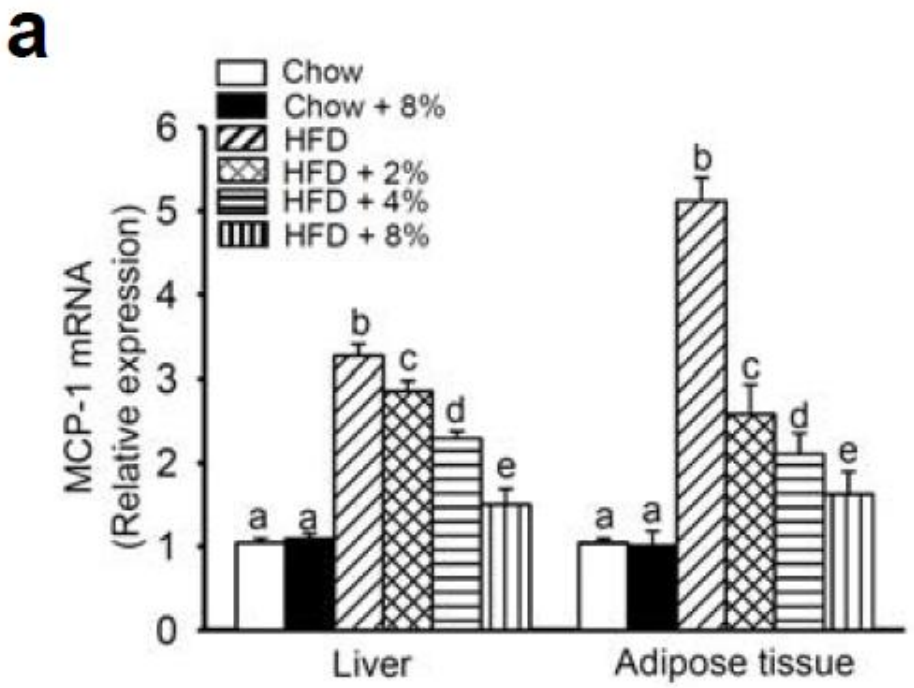
抗炎细胞因子

mRNA



血清促炎细胞因子

蛋白质



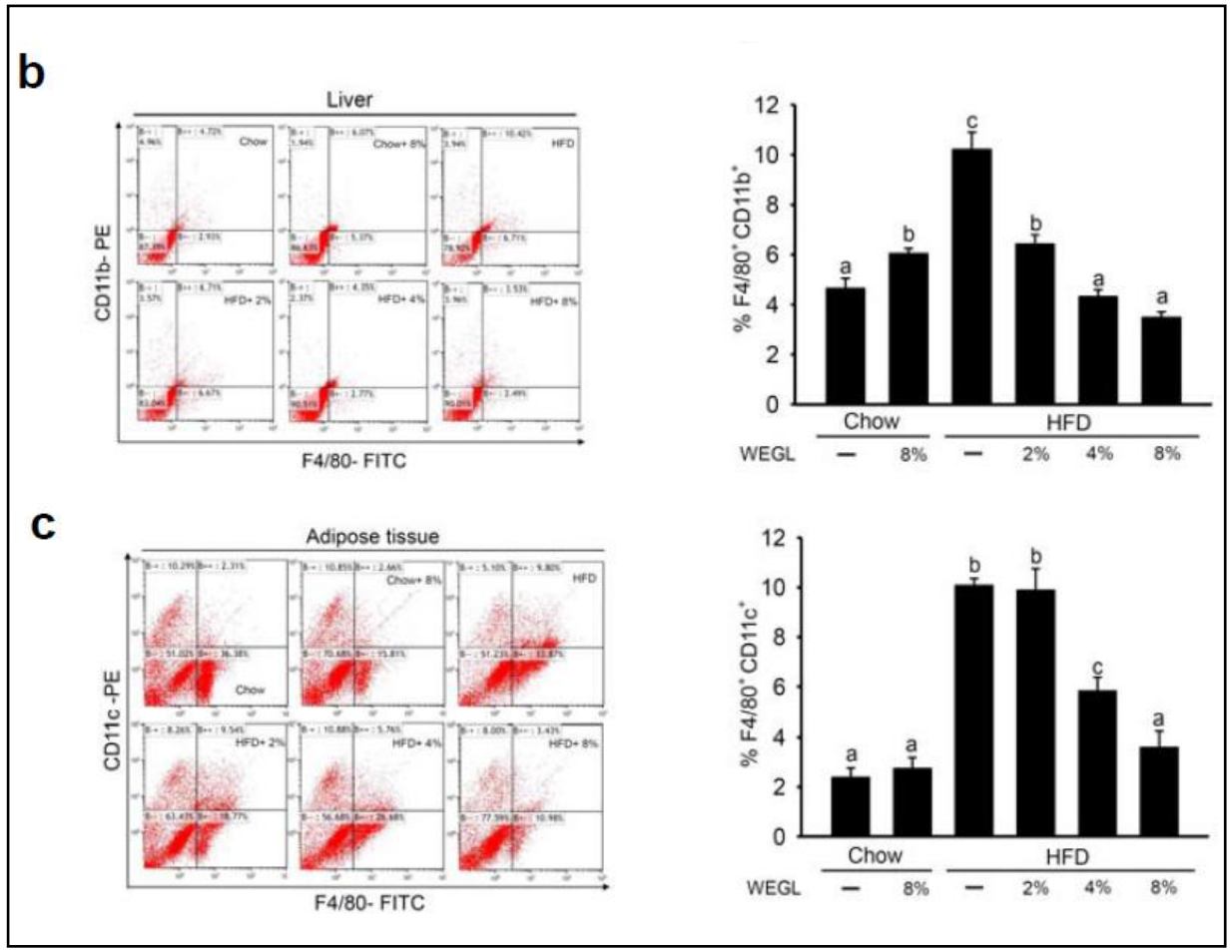
肥胖 ⟷ 肝脏和脂肪组织内的免疫细胞

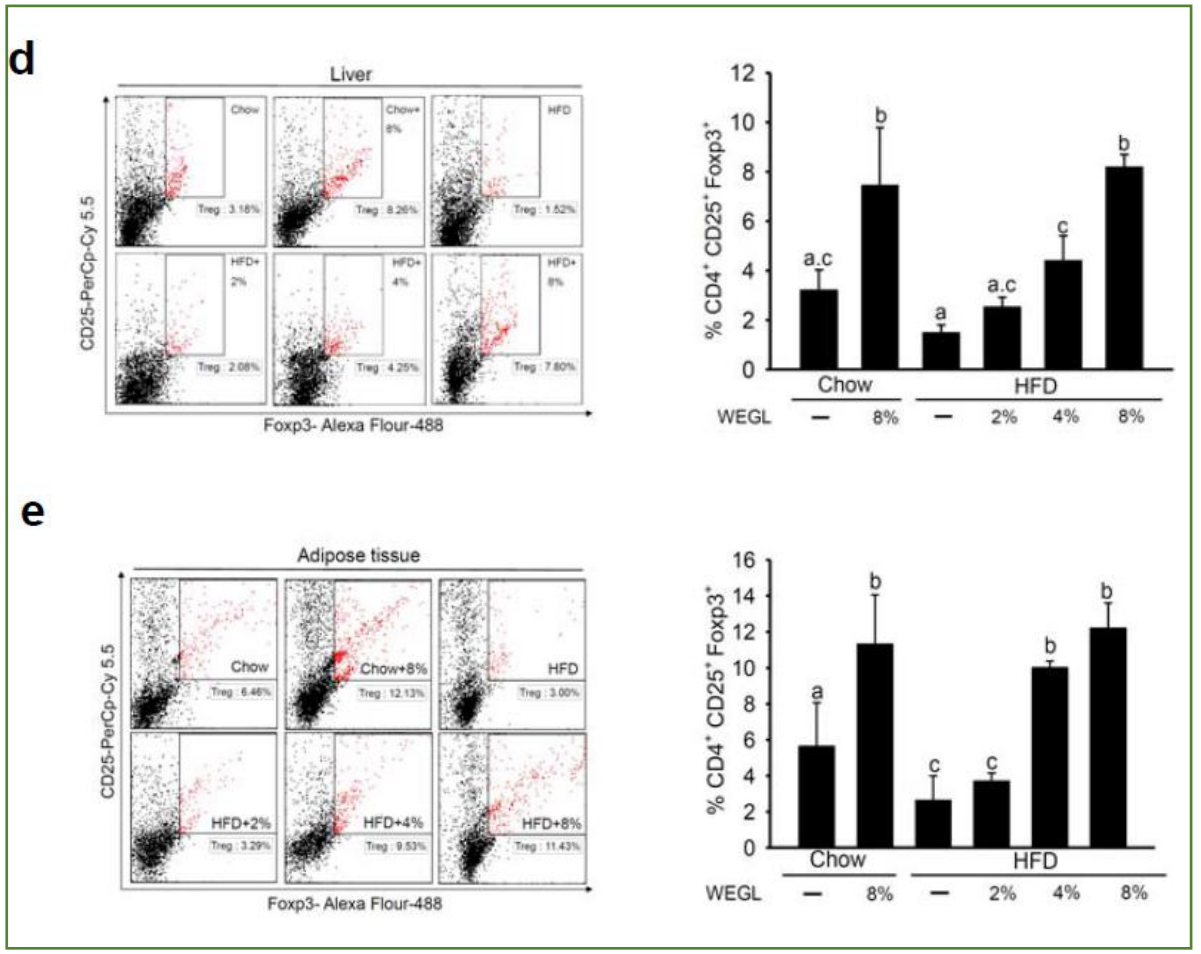
M1巨噬细胞

⇕

MCP-1

单核细胞趋化蛋白-1


b
Liver
Chow
Chow+ 8%
HFD
HFD+ 2%
HFD+ 4%
HFD+ 8%
CD11b- PE
F4/80- FITC
% F4/80+ CD11b+
Chow
HFD
WEGL
—
8%
2%
4%
c
Adipose tissue
CD11c -PE
% F4/80+ CD11c+


d
Liver
CD25-PerCp-Cy 5.5
Foxp3- Alexa Flour-488
% CD4+ CD25+ Foxp3+
e
Adipose tissue

巨噬细胞

T细胞

内毒素血症

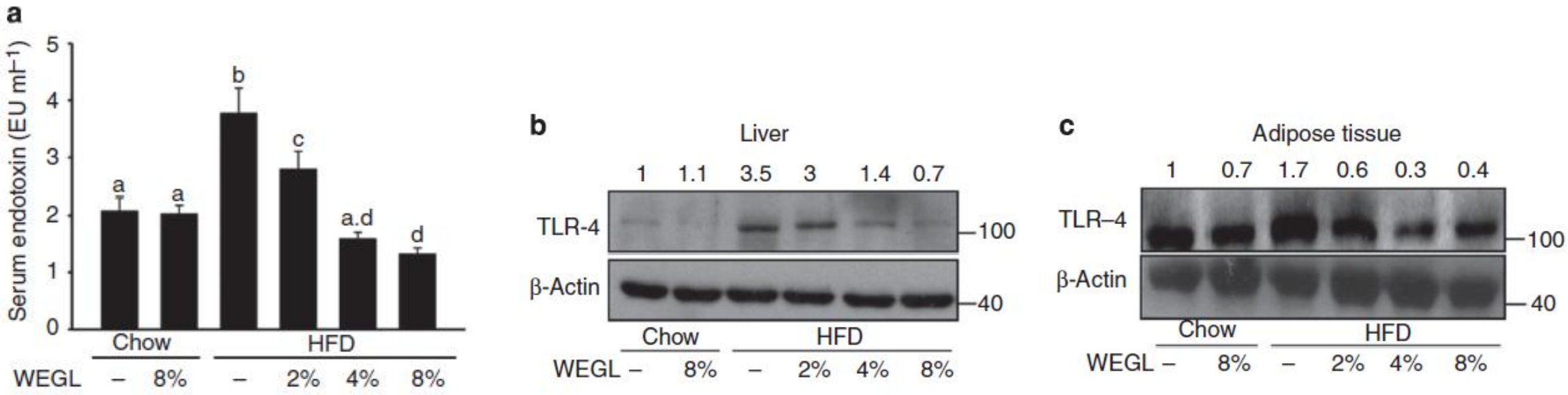
TLR4信号

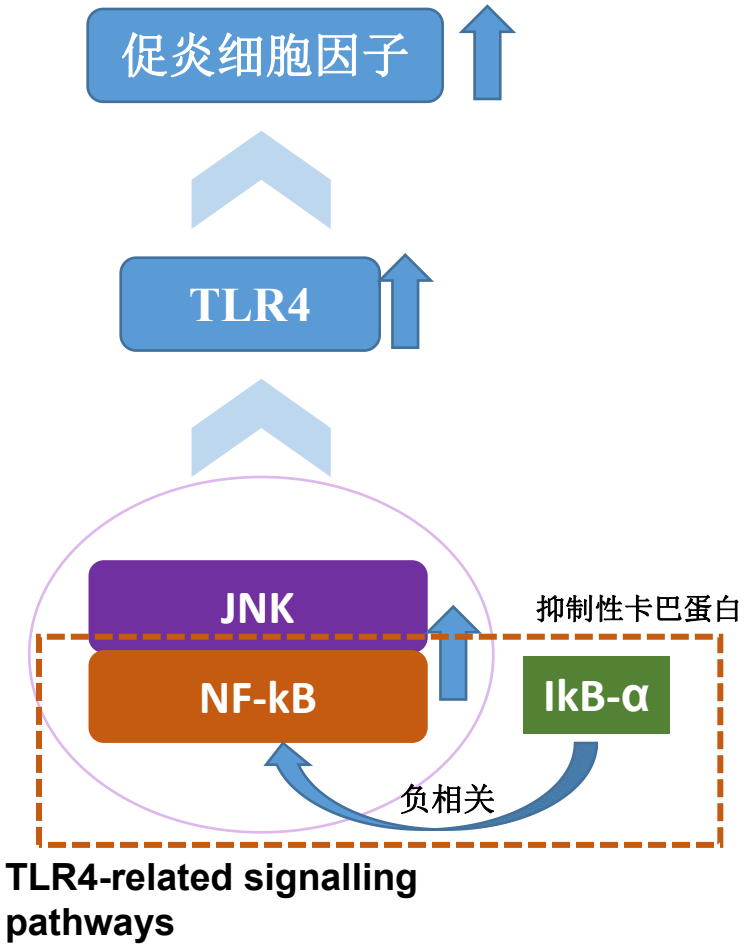
促炎细胞因子

慢性炎症

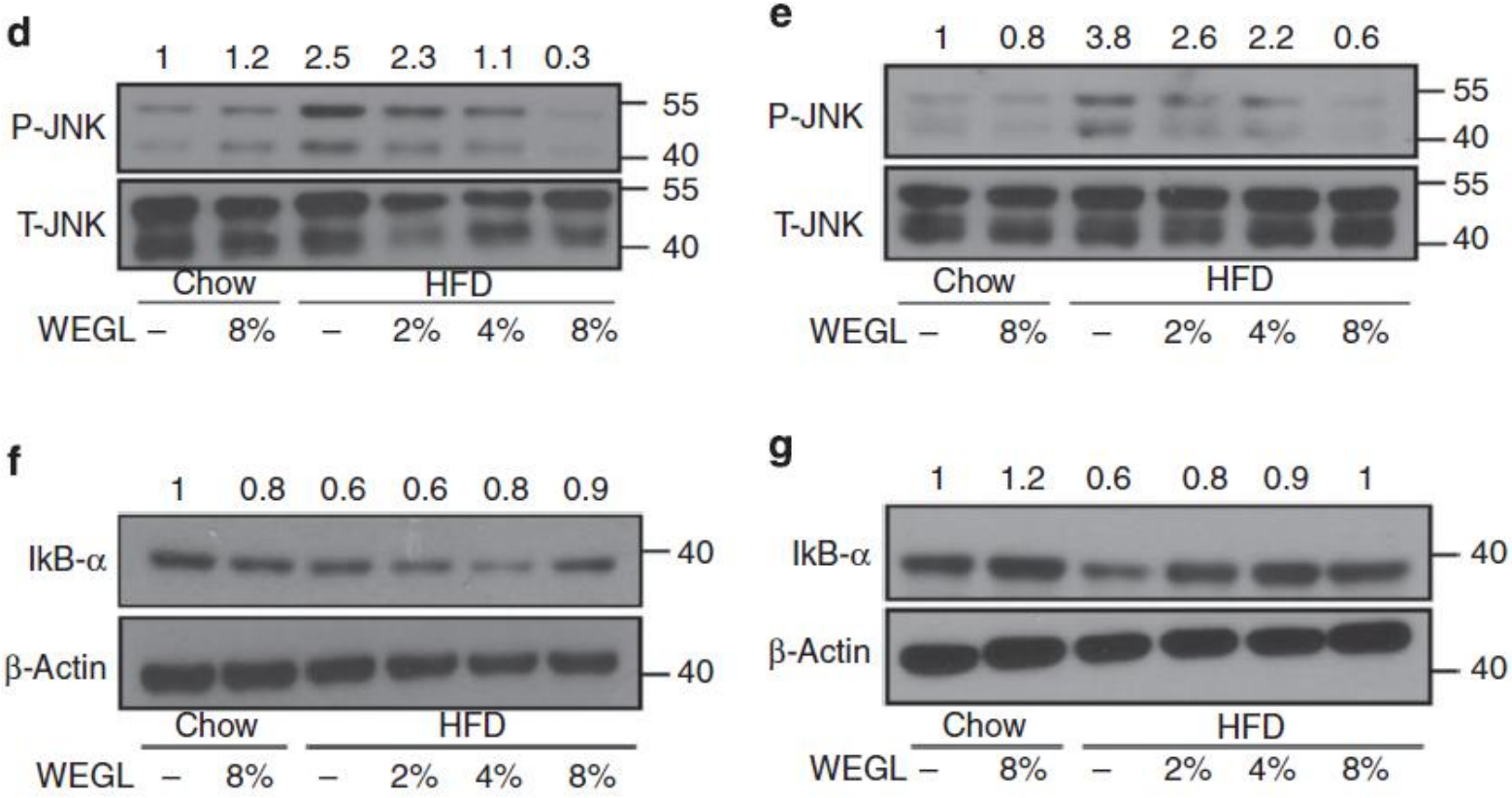
胰岛素抵抗

(Cani, P. D. *et al*., 2008)

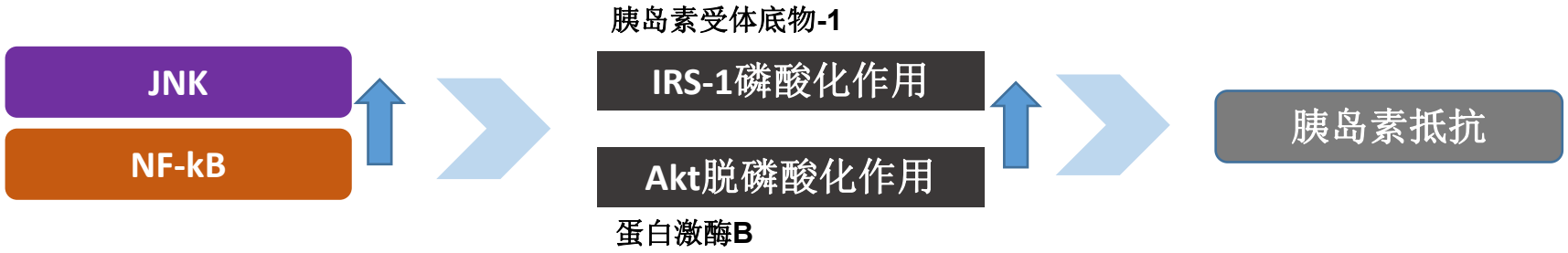




TLR4-related signalling pathways

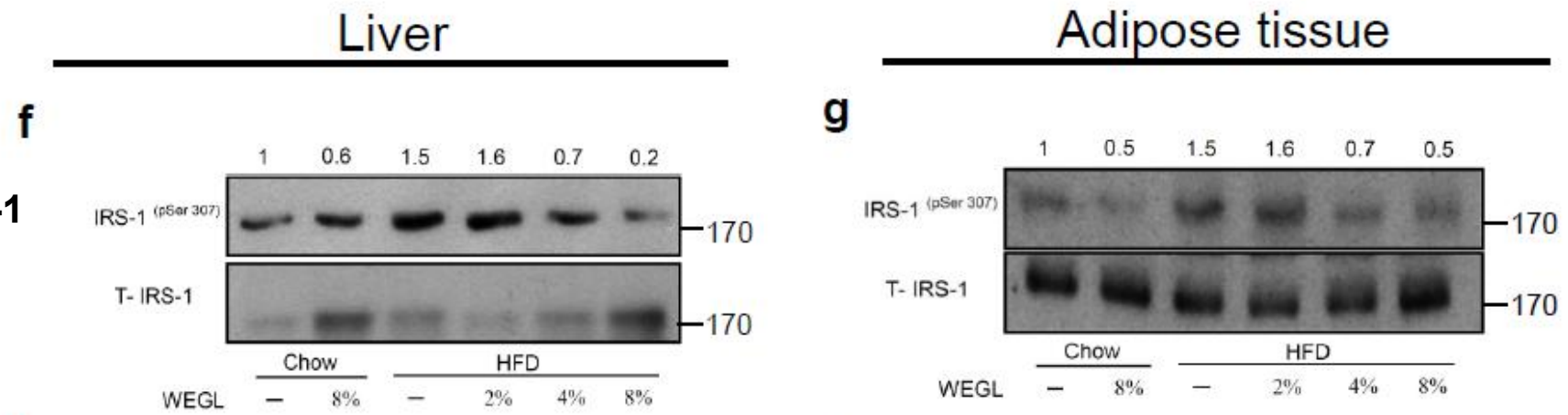


(Han, M. S. *et al*., 2013; Cai, D. *et al*., 2005)

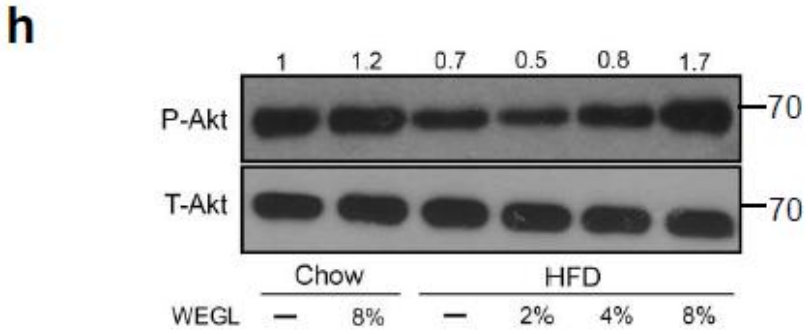


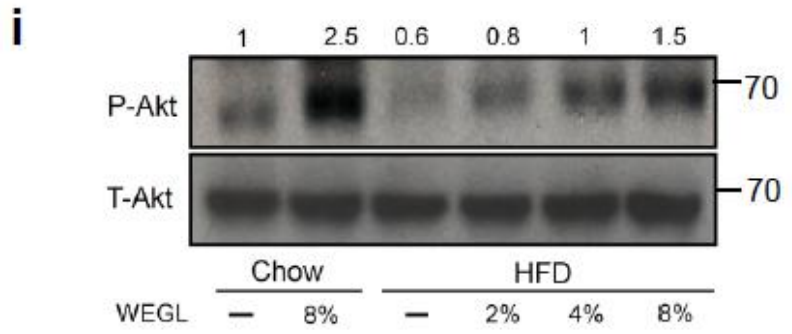
(Qatanani, M. & Lazar, M. A., 2007)

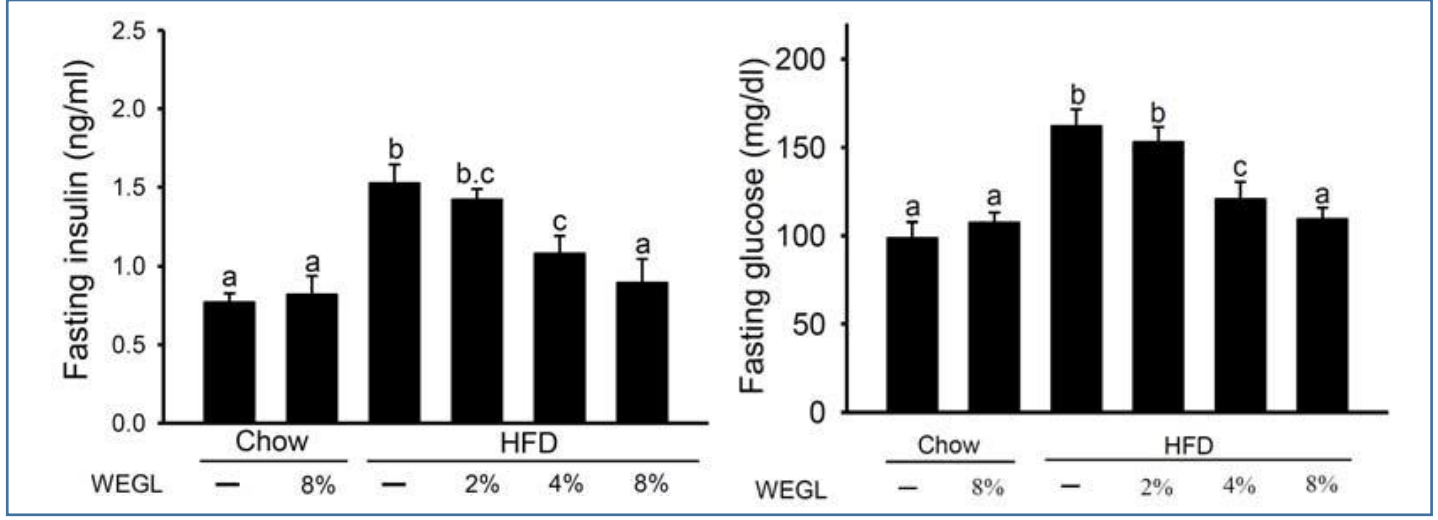
磷酸化IRS-1



磷酸化Akt

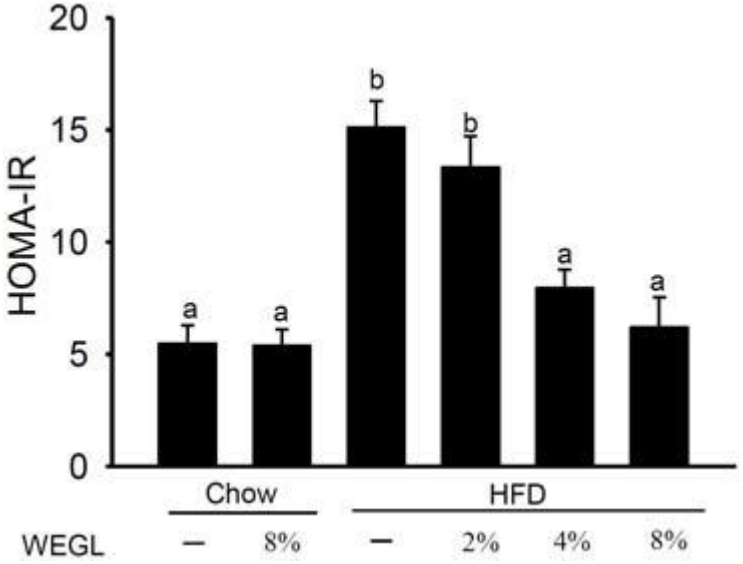


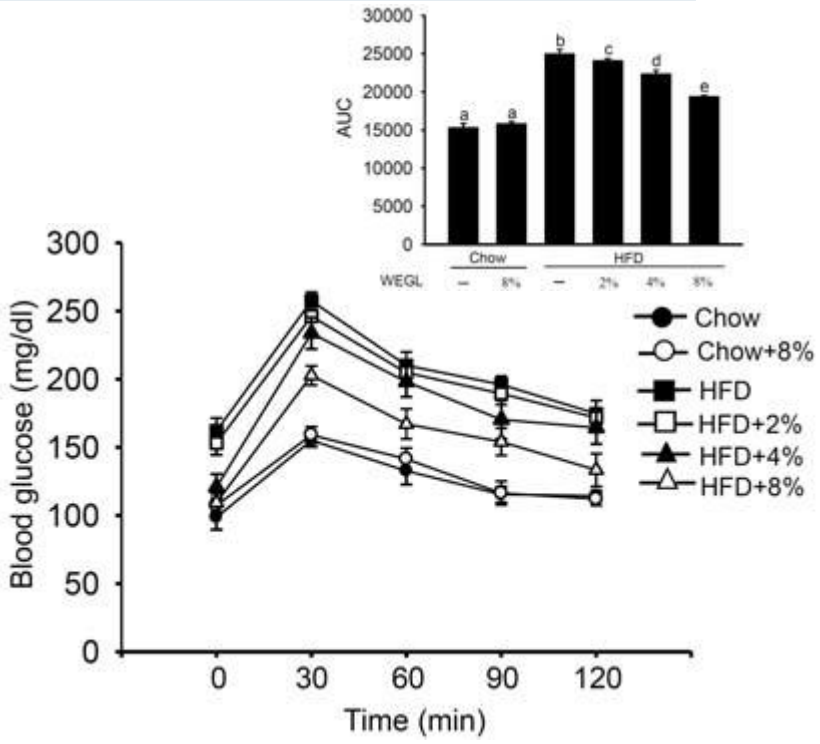




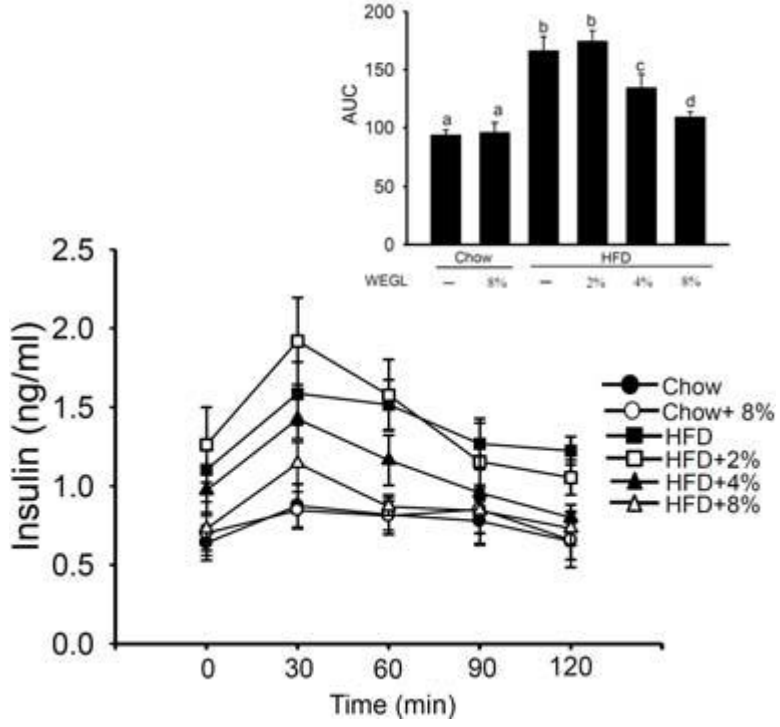
空腹血清胰岛素、葡萄糖糖水平

胰岛素抵抗指数



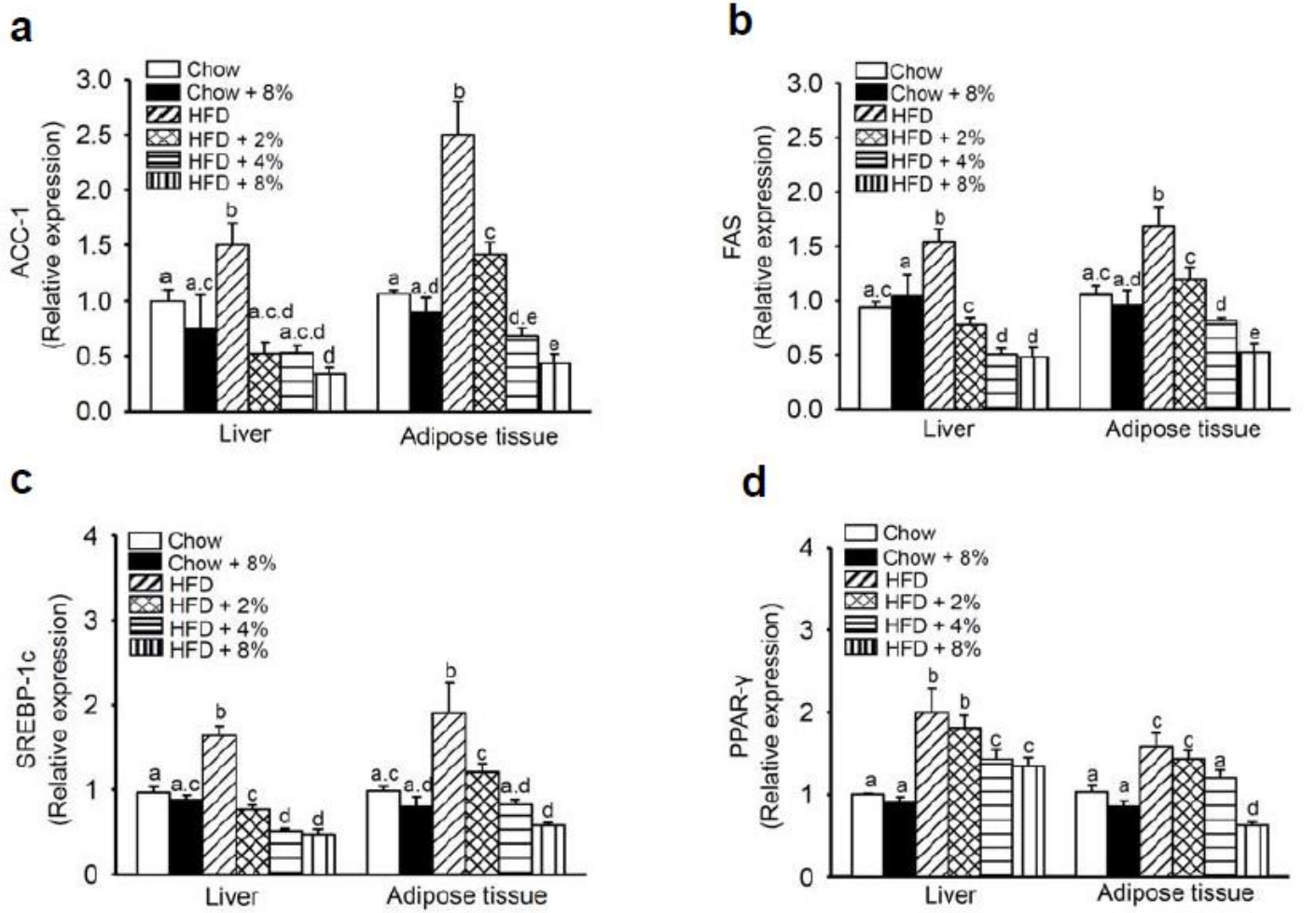


灌胃——葡萄糖耐受试验



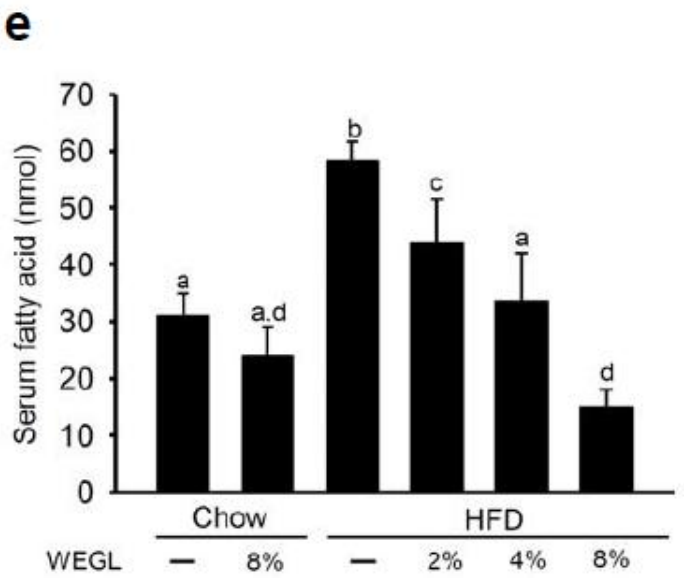
3 结果

4. WEGL regulates lipogenic gene expression.



肝脏、脂肪组织

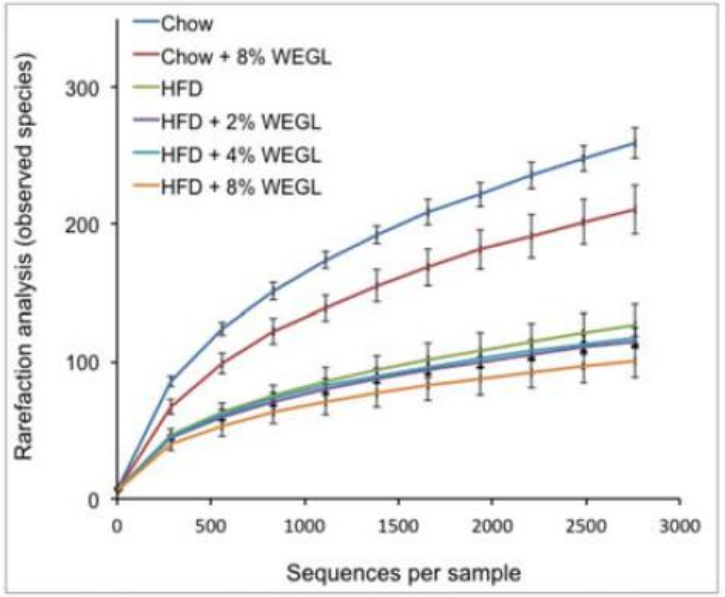
脂肪生成相关基因



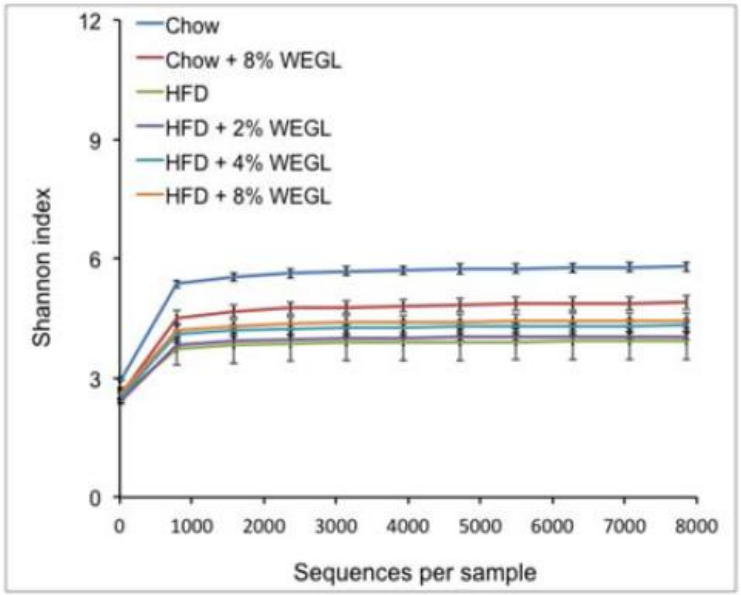
3 结果

5. WEGL reverses HFD-induced gut dysbiosis. 肠道菌群

a



b



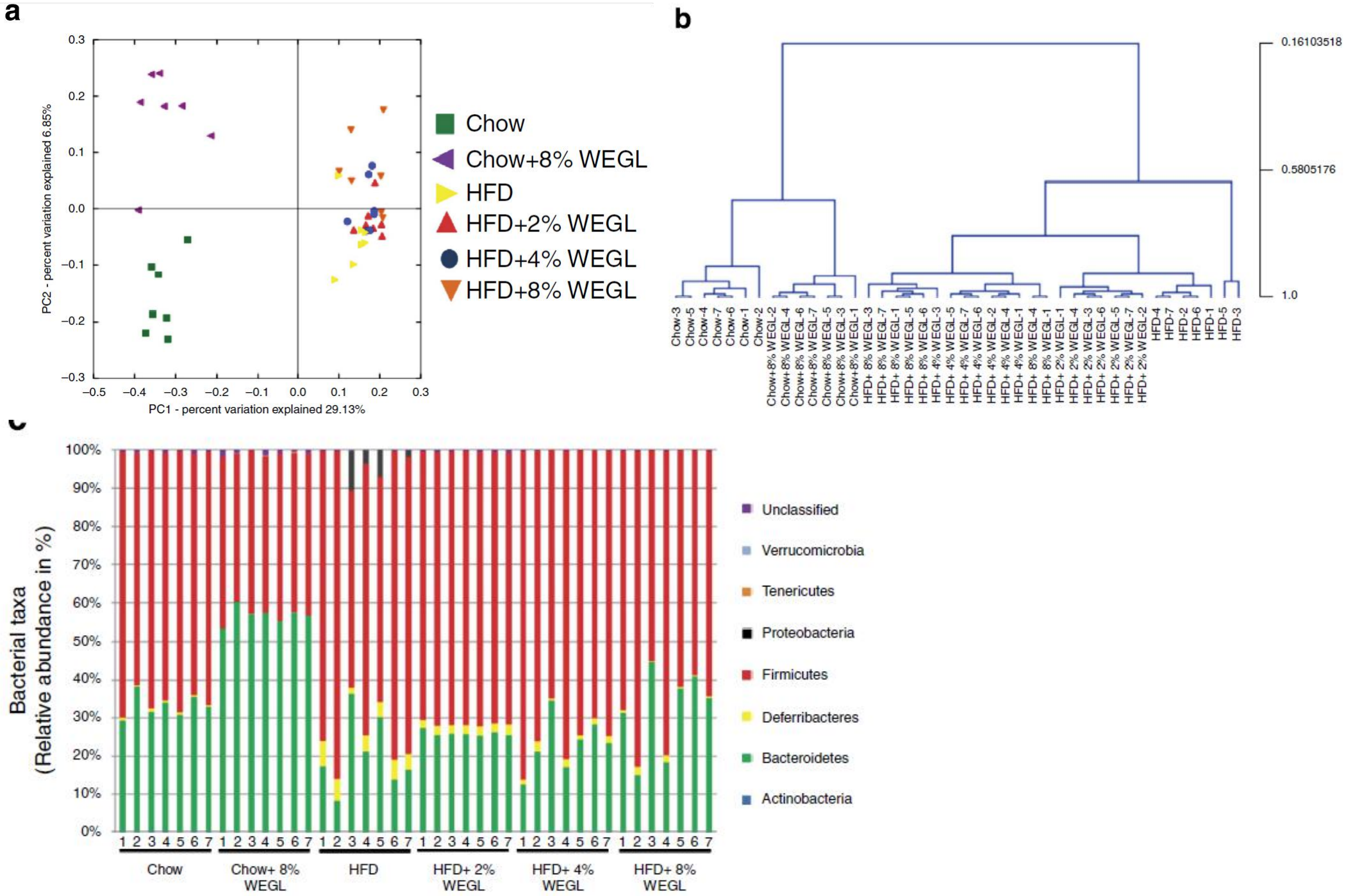
691,370 raw reads

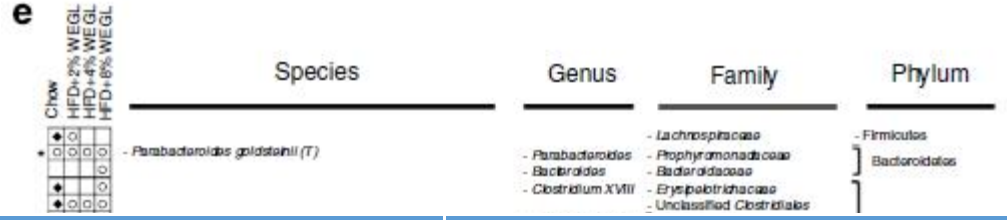
16,461±5,411 reads per sample

292,952 effective reads

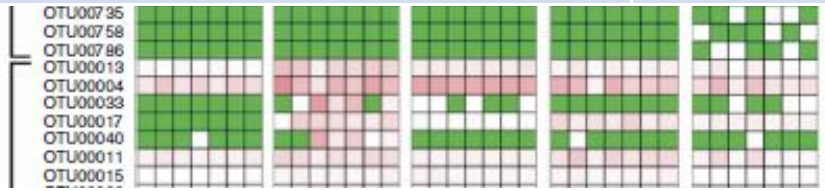
6,975±2,192 effective reads per sample

(n=7 for each group)


a
PC2 - percent variation explained 6.85%
PC1 - percent variation explained 29.13%
Chow
Chow+8% WEGL
HFD
HFD+2% WEGL
HFD+4% WEGL
HFD+8% WEGL
b
0.16103518
0.5805176
1.0
c
Bacterial taxa
(Relative abundance in %)
Unclassified
Verrucomicrobia
Tenericutes
Proteobacteria
Firmicutes
Deferribacteres
Bacteroidetes
Actinobacteria
Chow
Chow+ 8% WEGL
HFD
HFD+ 2% WEGL
HFD+ 4% WEGL
HFD+ 8% WEGL

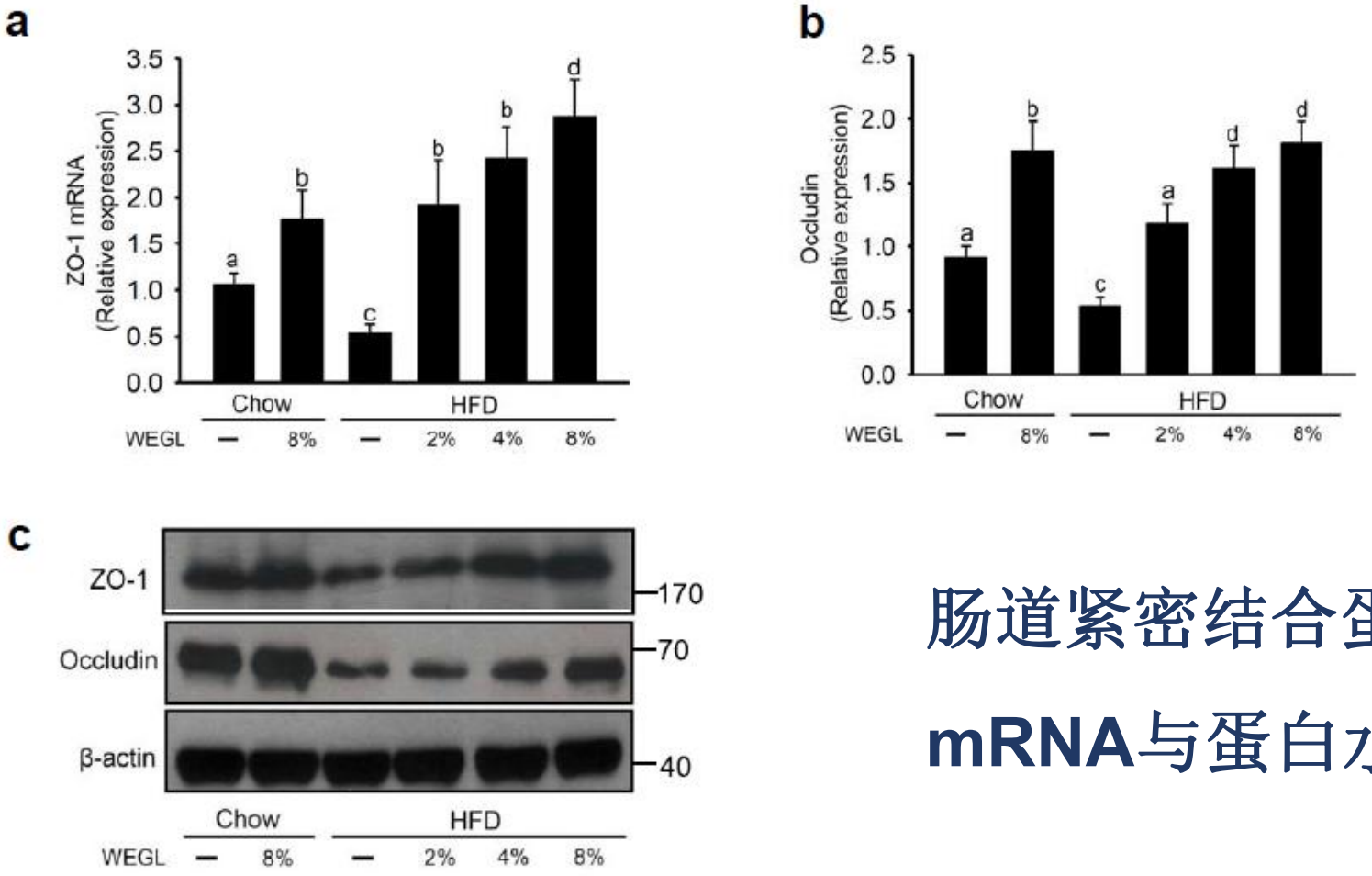


HFD组增加而8% WEGL组减少	8% WEGL组较HFD组增加	Chow+8% WEGL组较HFD组增加
Mucispirilum shaedleri	*Parabacteroides goldsteinii*	*E.coprostanoligenes*
Escherichia fergusonii	***Bacteroides* spp.**	*C. methylpentosum*
Enterococcus spp.	*Roseburia hominis*	*P. goldsteinii*
Lactococcus lactis	*Anaerotruncus colihominis*	*Bacteroides* spp.
Clostridium lactatifermentans(Clostridium XIVb)	***Clostridium methylpentosum*(Clostridium IV)**	*A. colihominis*
Oscillibacter valericigenes	***Clostridium* XIVa and XVIII**	*R. hominis*
(已有报道的肥胖相关细菌)	*Eubacterium coprostanoligenes*	*Clostridium* XIVa and XVIII



3 结果

6. WEGL maintains intestinal integrity in HFD mice.



肠道紧密结合蛋白

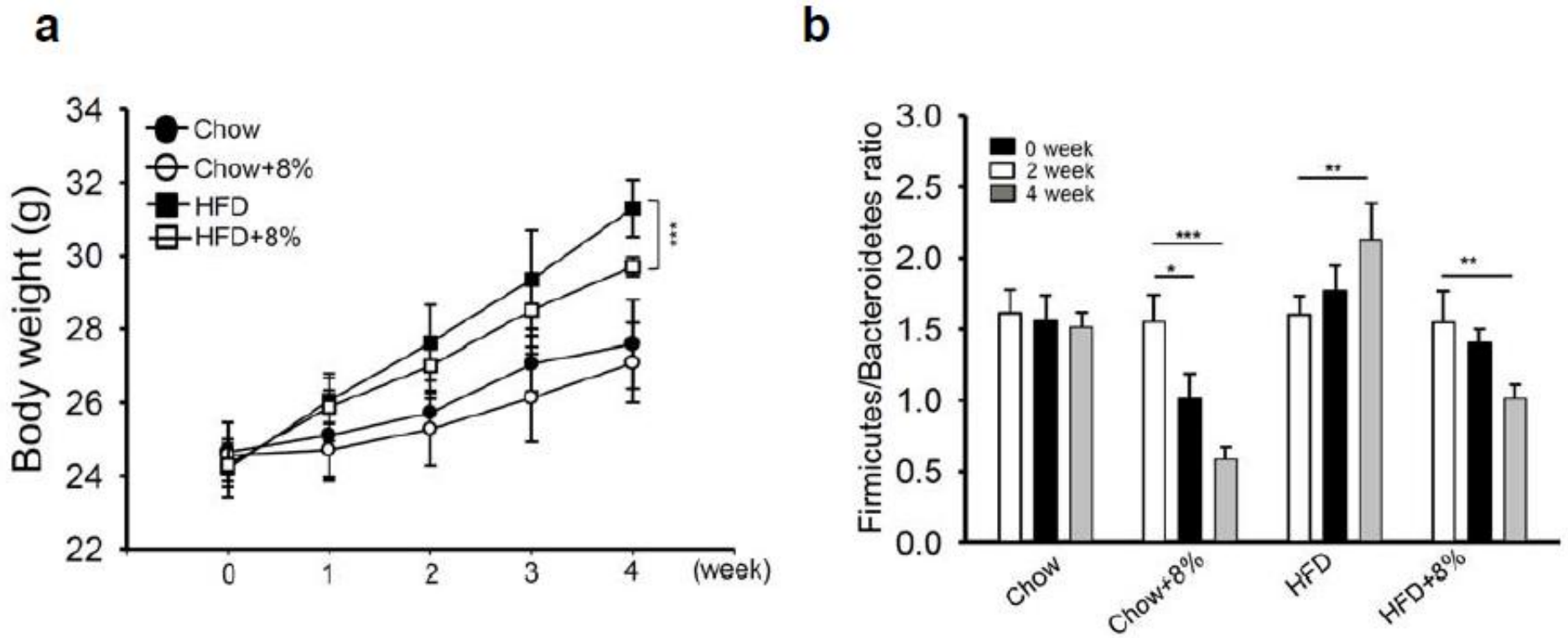
mRNA与蛋白水平表达量

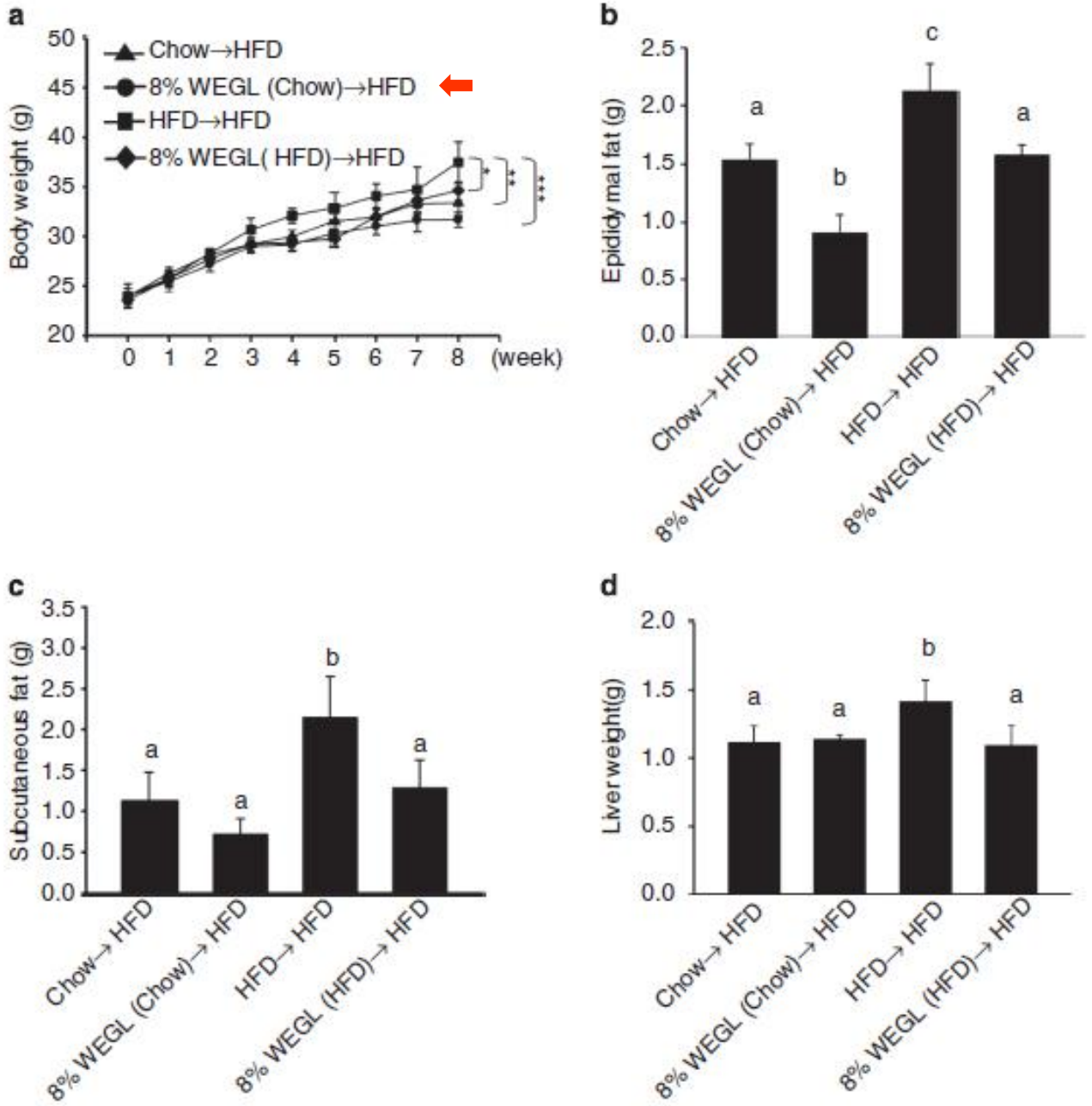
3 结果

粪菌移植

7. **WEGL faecal transplants reduce obesity.**

供体小鼠（**4**周）





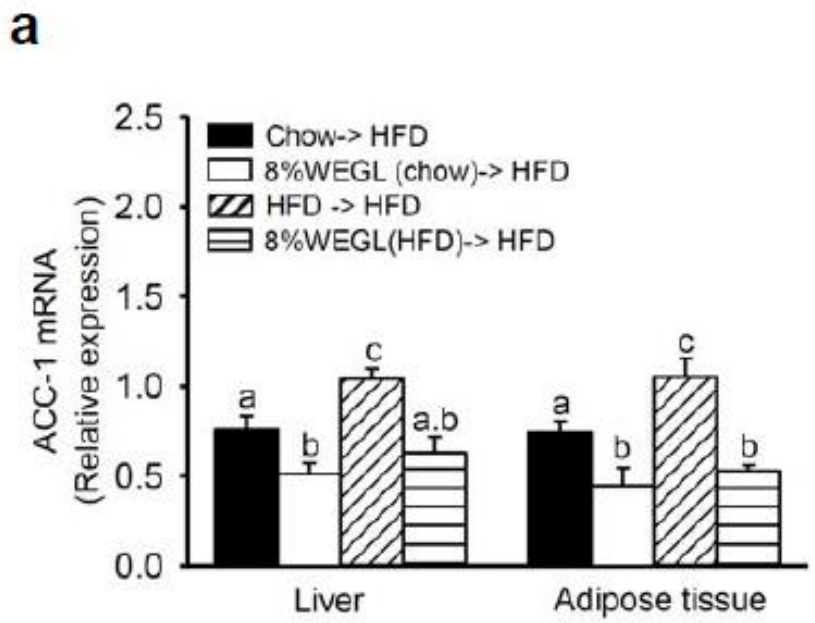
受体小鼠

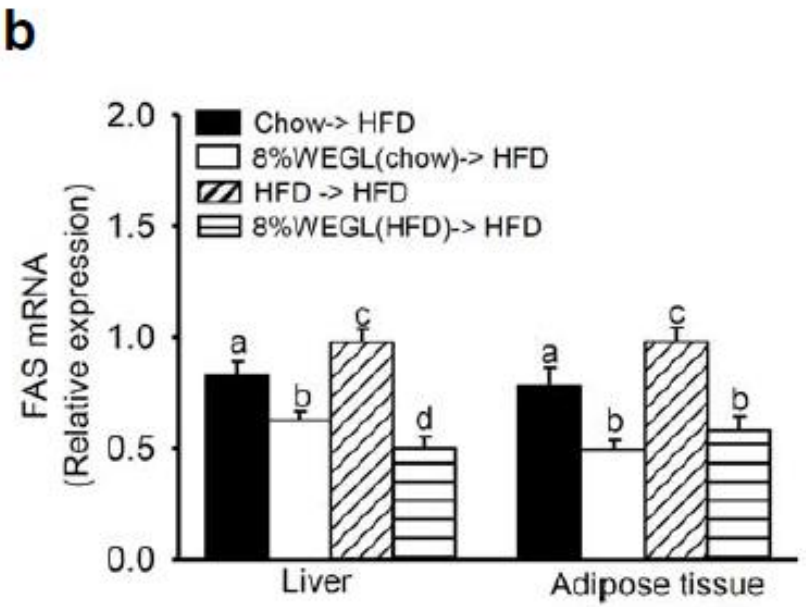
体重

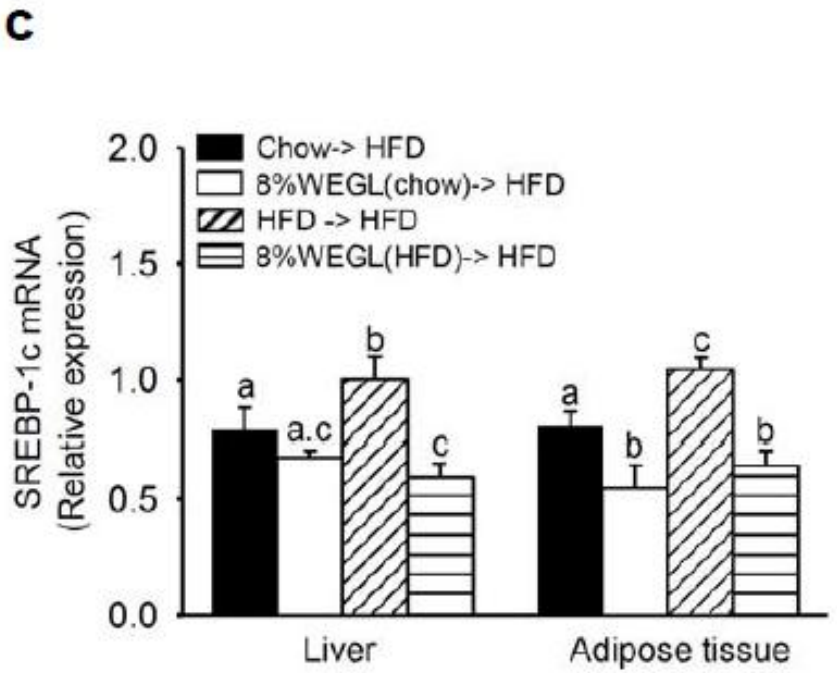
附睾脂肪

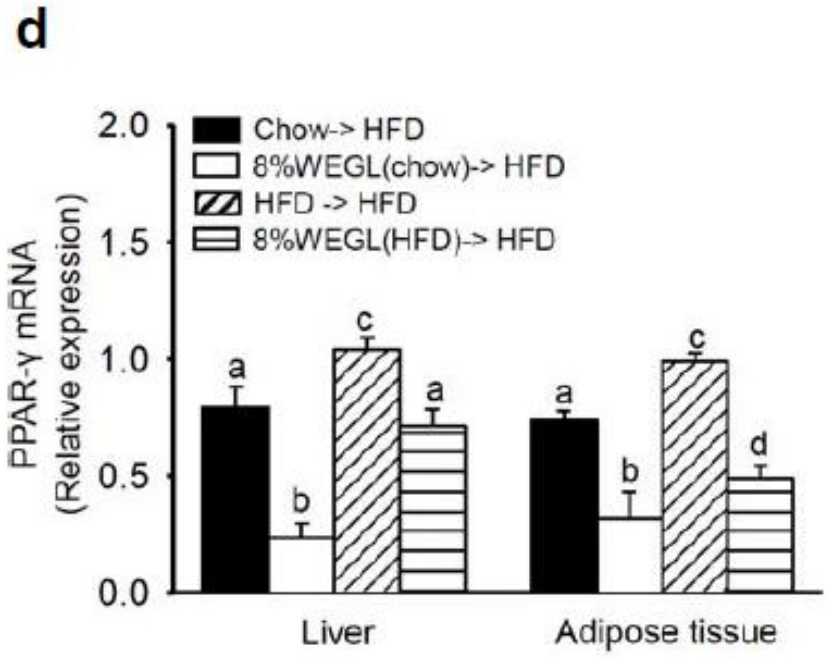
皮下脂肪

肝脏重量





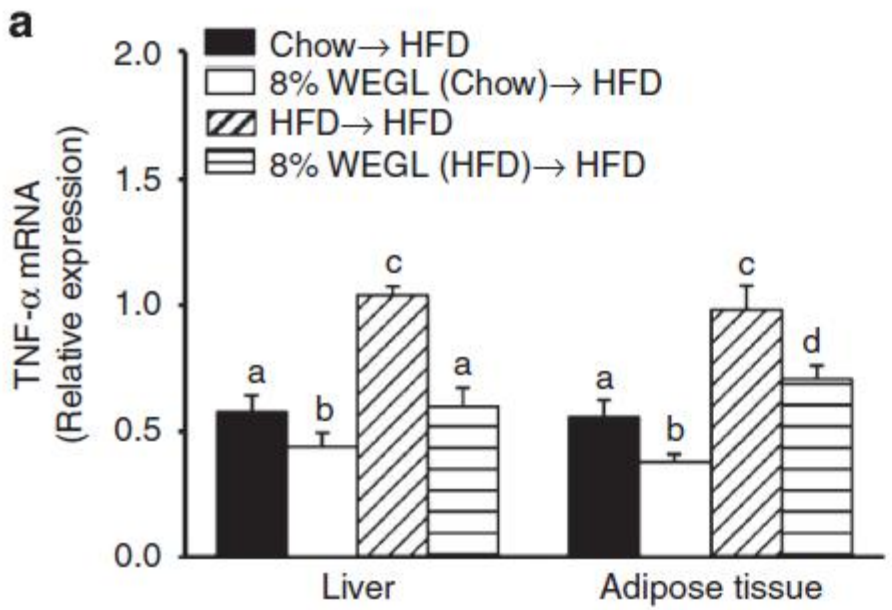


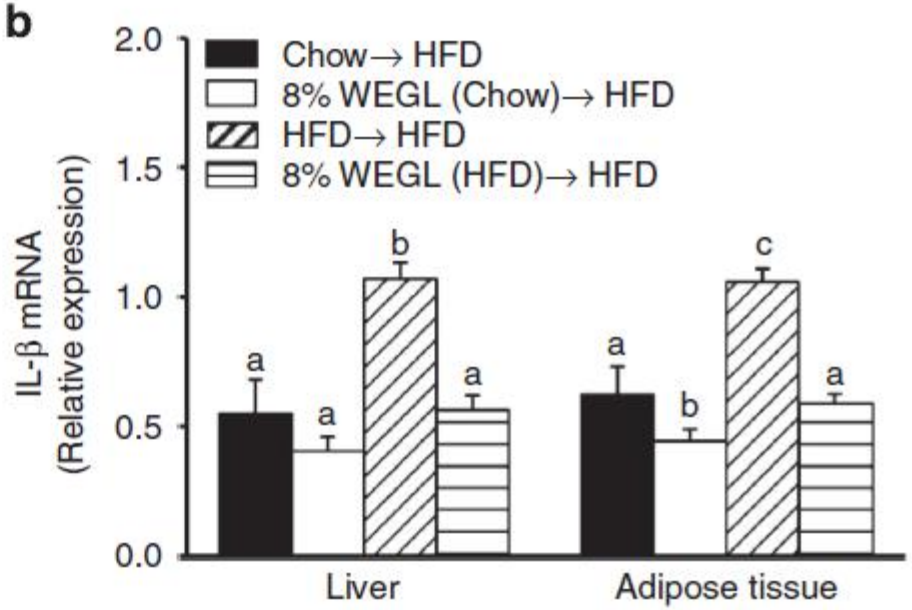


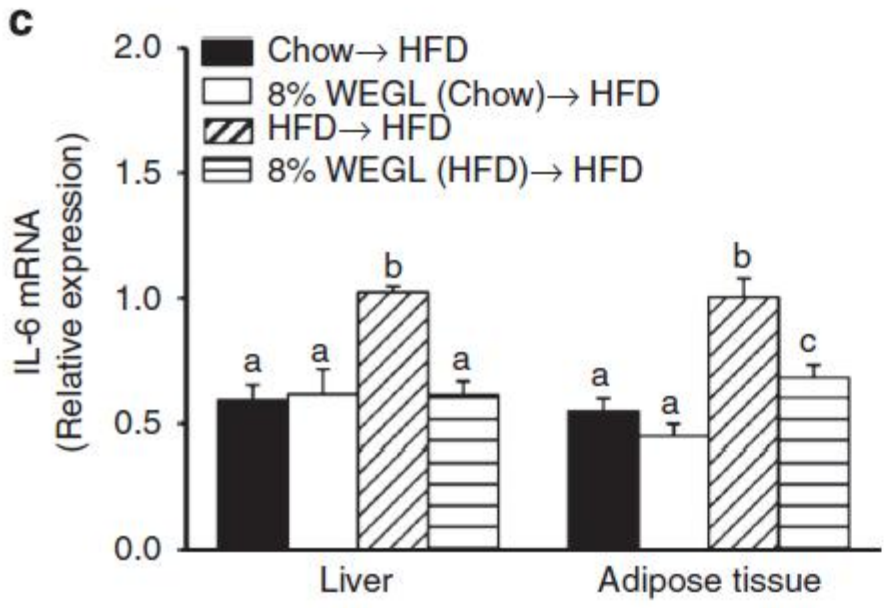
受体小鼠

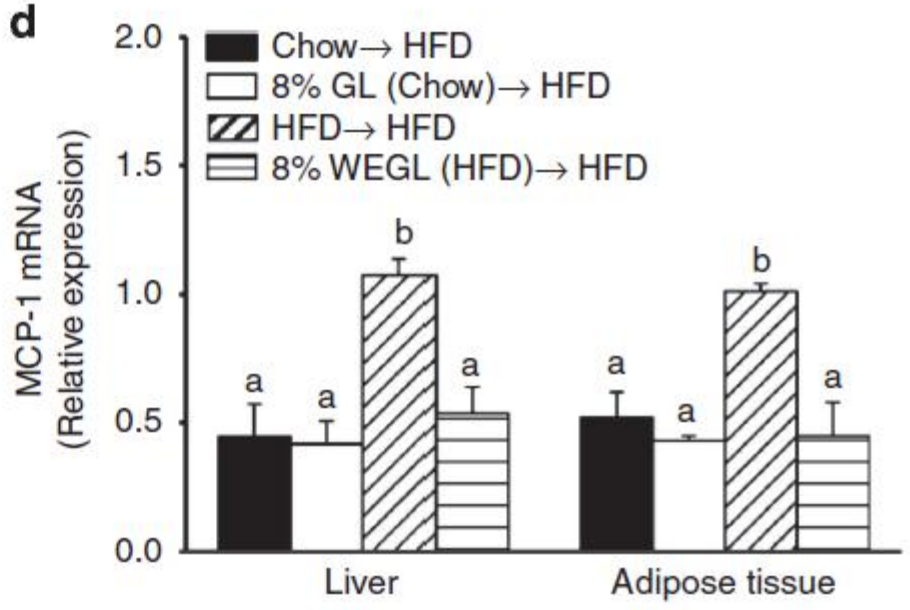
肝脏、脂肪组织

脂肪生成相关基因





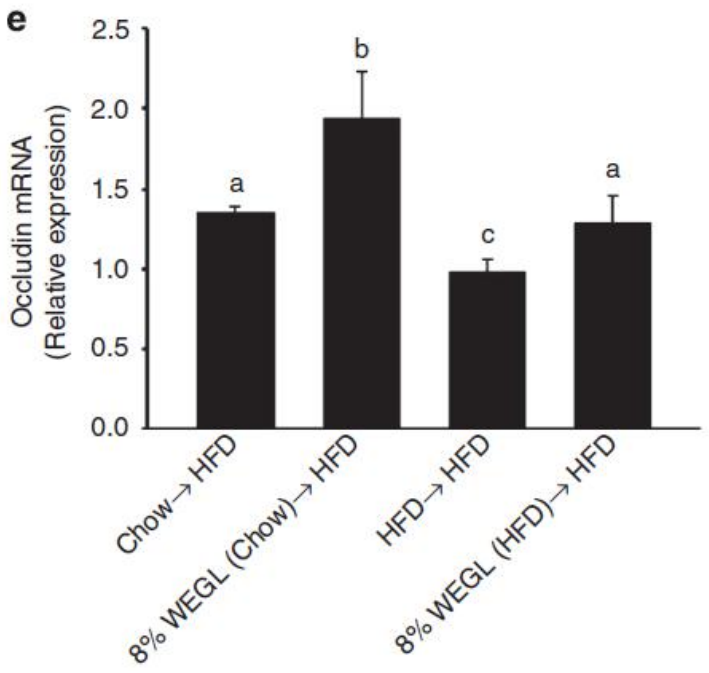


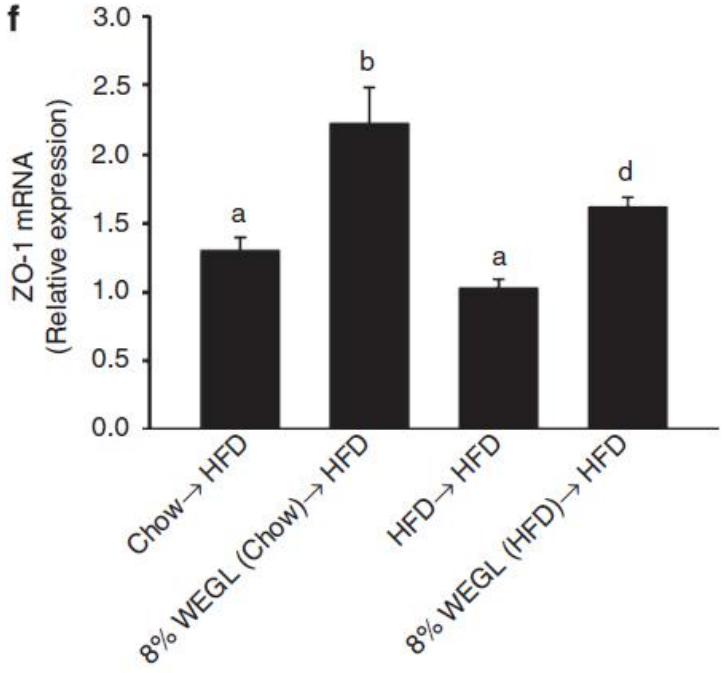


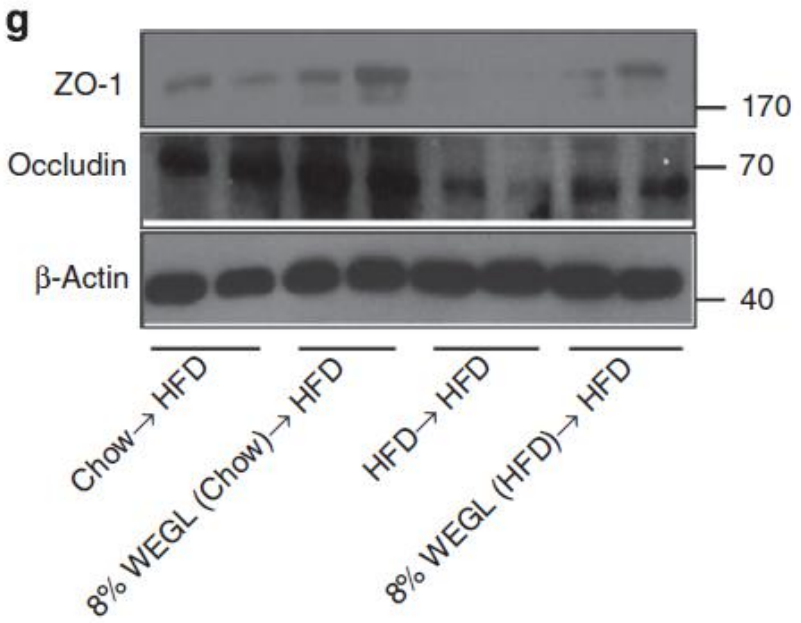
受体小鼠

炎症细胞因子

单核细胞趋化蛋白-1







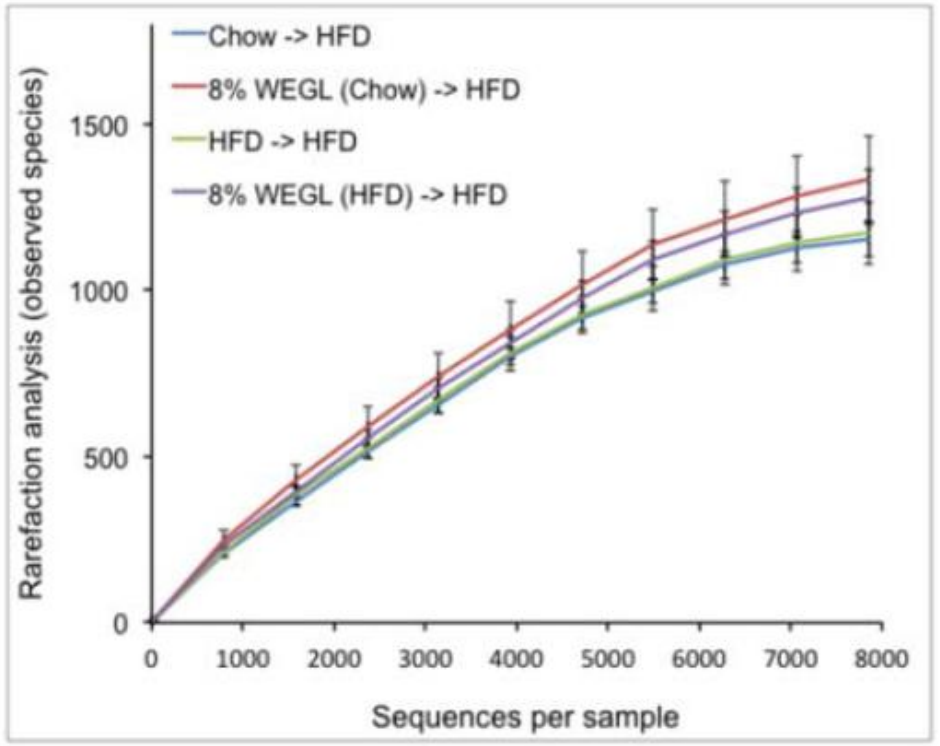
受体小鼠

肠道紧密结合蛋白

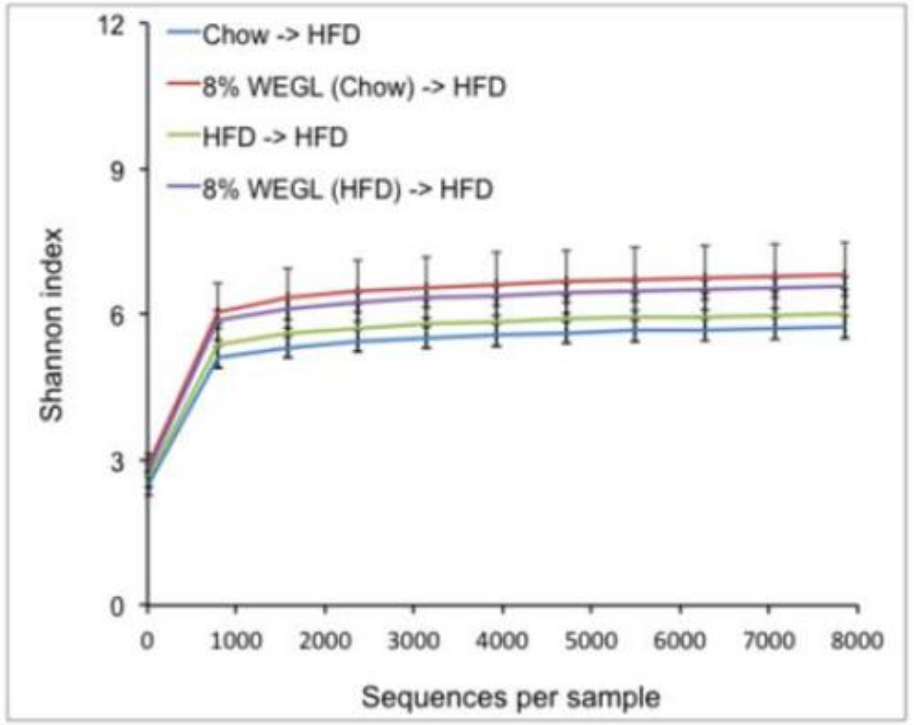
3 结果

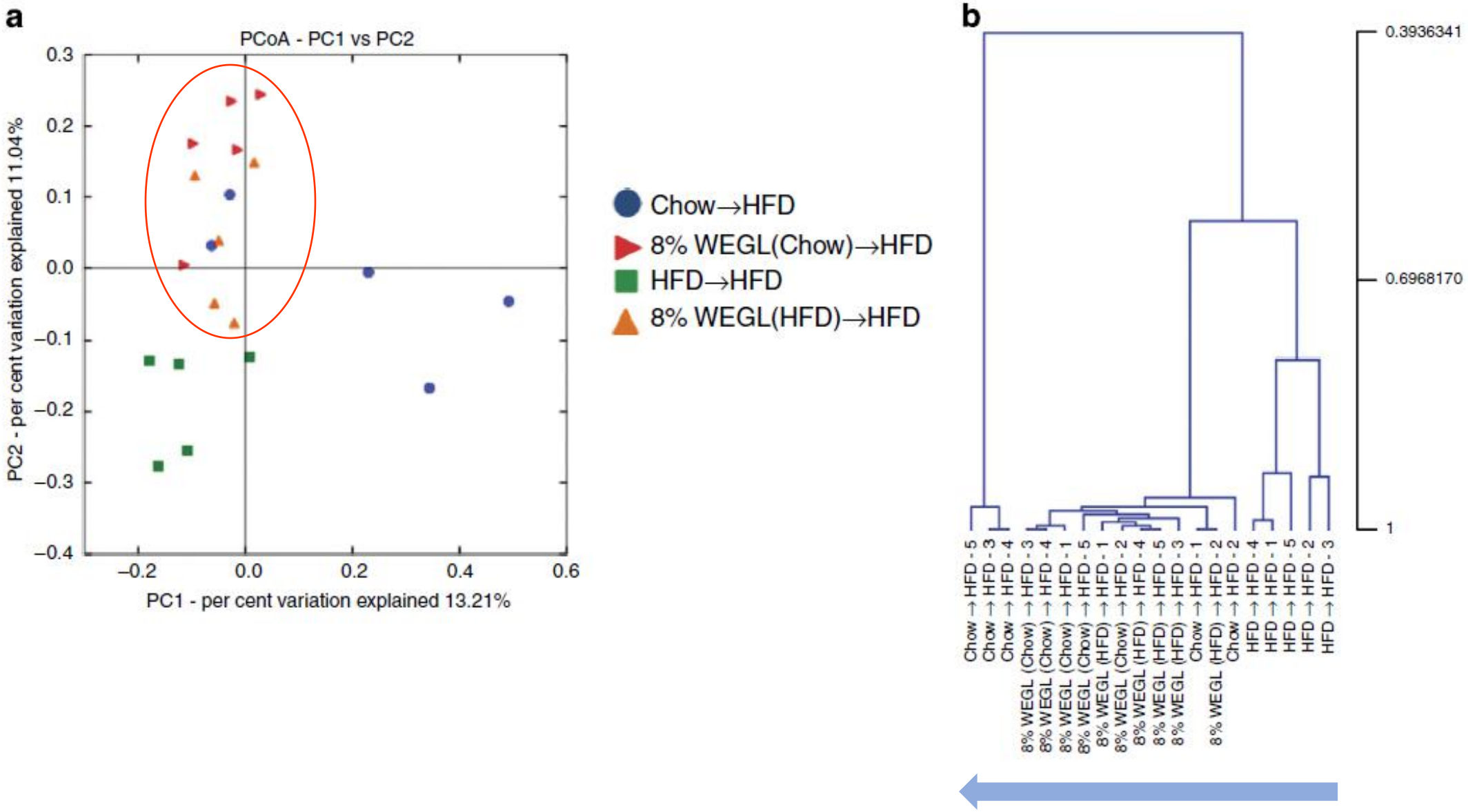
7. WEGL faecal transplants modulate gut microbiota composition

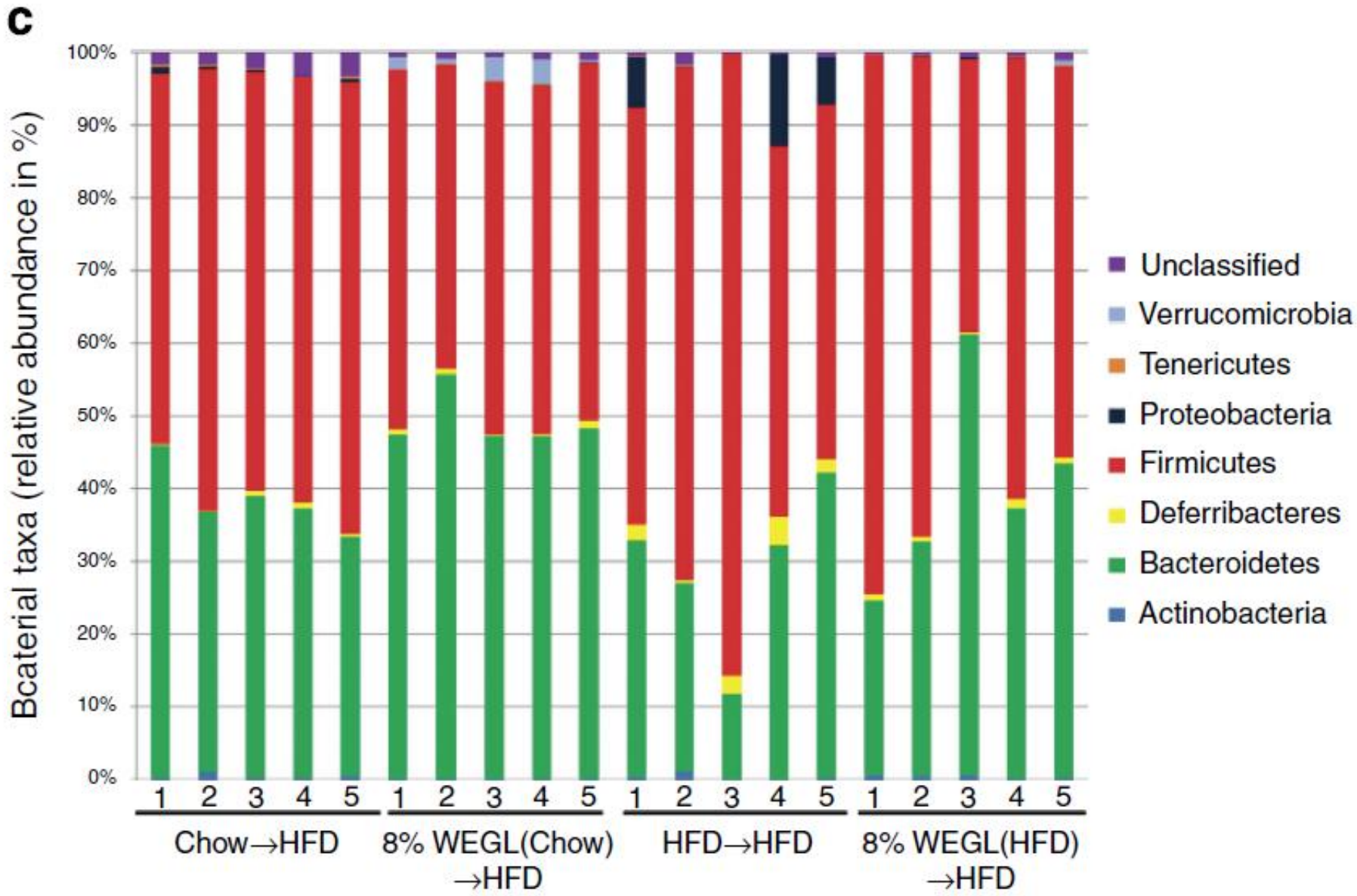
a

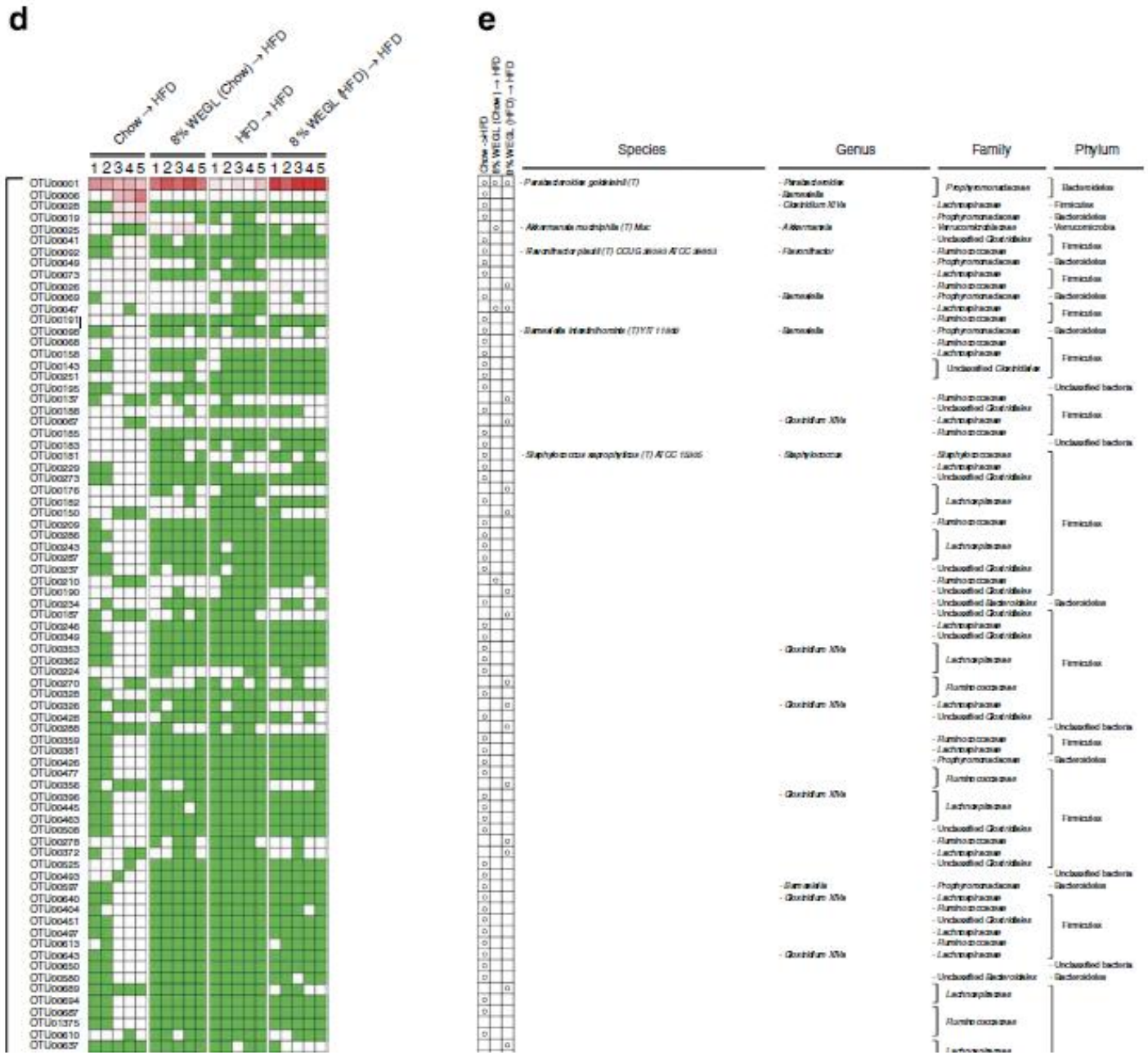


b




a
PCoA - PC1 vs PC2
PC2 - per cent variation explained 11.04%
PC1 - per cent variation explained 13.21%
0.3
0.2
0.1
0.0
−0.1
−0.2
−0.3
−0.4
−0.2
0.0
0.2
0.4
0.6
Chow→HFD
8% WEGL(Chow)→HFD
HFD→HFD
8% WEGL(HFD)→HFD
b
0.3936341
0.6968170
1
Chow → HFD - 5
Chow → HFD - 3
Chow → HFD - 4
8% WEGL (Chow) → HFD - 3
8% WEGL (Chow) → HFD - 4
8% WEGL (Chow) → HFD - 1
8% WEGL (Chow) → HFD - 5
8% WEGL (HFD) → HFD - 1
8% WEGL (Chow) → HFD - 2
8% WEGL (HFD) → HFD - 4
8% WEGL (HFD) → HFD - 5
8% WEGL (HFD) → HFD - 3
Chow → HFD - 1
8% WEGL (HFD) → HFD - 2
Chow → HFD - 2
HFD → HFD - 4
HFD → HFD - 1
HFD → HFD - 5
HFD → HFD - 2
HFD → HFD - 3


c
Bcaterial taxa (relative abundance in %)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1 2 3 4 5 1 2 3 4 5 1 2 3 4 5 1 2 3 4 5
Chow→HFD
8% WEGL(Chow) →HFD
HFD→HFD
8% WEGL(HFD) →HFD
Unclassified
Verrucomicrobia
Tenericutes
Proteobacteria
Firmicutes
Deferribacteres
Bacteroidetes
Actinobacteria


d
Chow → HFD
8% WEGL (Chow) → HFD
HFD → HFD
8% WEGL (HFD) → HFD
1 2 3 4 5 1 2 3 4 5 1 2 3 4 5 1 2 3 4 5
e
Species
Genus
Family
Phylum

3 结果

7. WEGL high molecular weight polysaccharides reduce obesity.

灵芝中的高分子量多糖组成、多糖（分子量大于**300kDa** ）的单糖组成

Table 1 | Molecular weight analysis of polysaccharide subfractions isolated from WEGL mycelium.

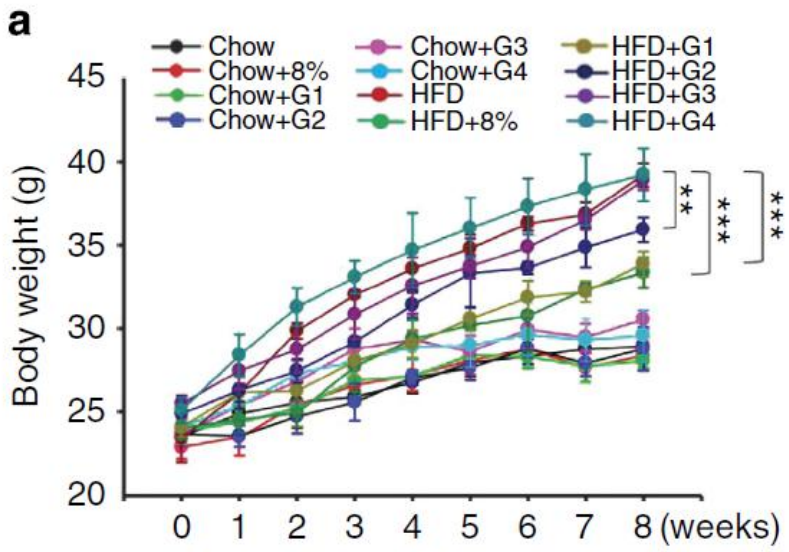
Subfraction	Component	Molecular weight (MW)	Percentage (%)
G1	Polysaccharide	>300 kDa	33.7
G2	Polysaccharide	10–300 kDa	15.6
G3	Polysaccharide	<10 kDa	4.0
G4	Mono-, di-, oligosaccharide	Undetermined	46.8

Polysaccharides were analysed from a 100-ml solution of 20% WEGL (w/v).

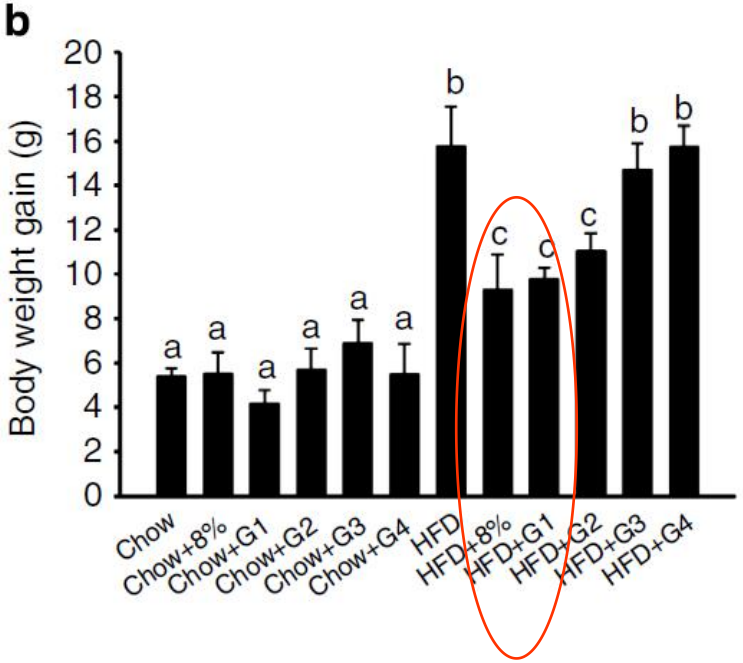
Table 2 | Monosaccharide composition of WEGL-G1 subfraction (>300 kDa).

	Man	Glc	Gal	GlcN	Ara	GalN	Rha	Fuc
Concentration ($mg\,l^{-1}$)	19.16	10.6	6.82	0.44	1.17	ND	0.99	1.18
Percentage (%)	47.5	26.3	16.9	1.1	2.9	ND	2.5	2.9

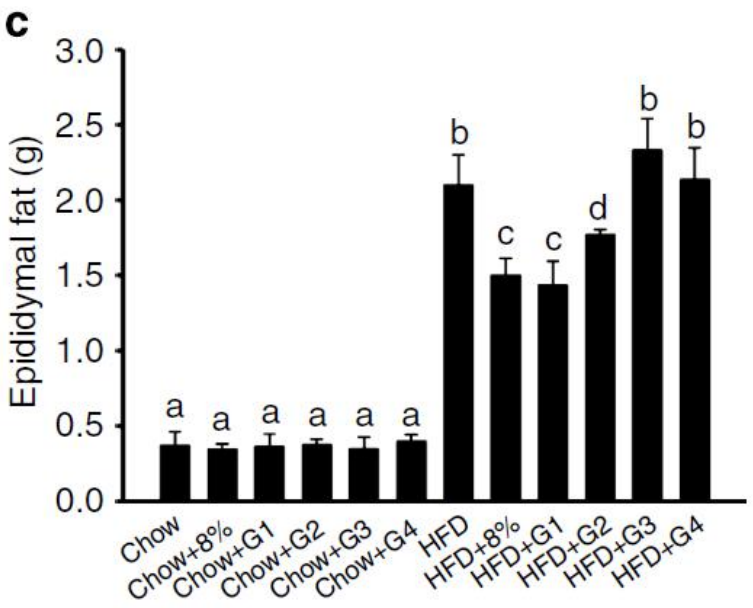
Ara, arabinose; Fuc, fucose; Gal, galactose; GalN, galactosamine; Glc, glucose; GlcN, glucosamine; Man, mannose; ND, not detected; Rha, rhamnose.



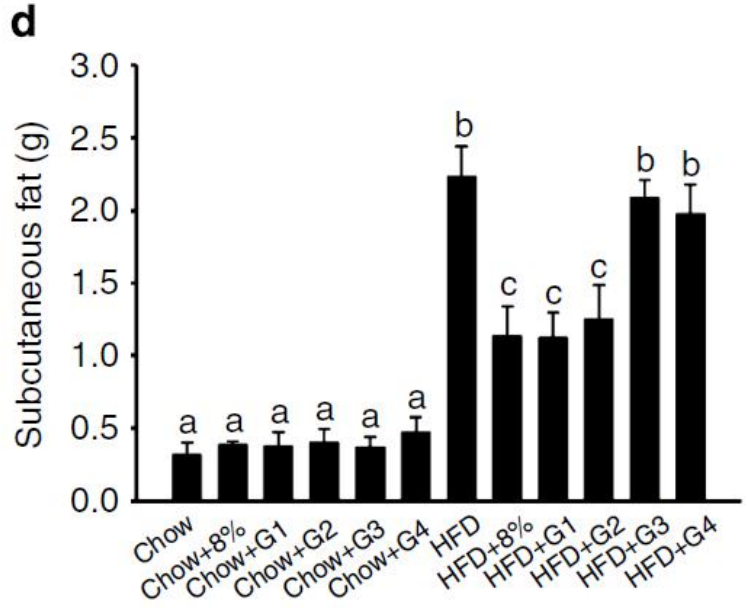
体重



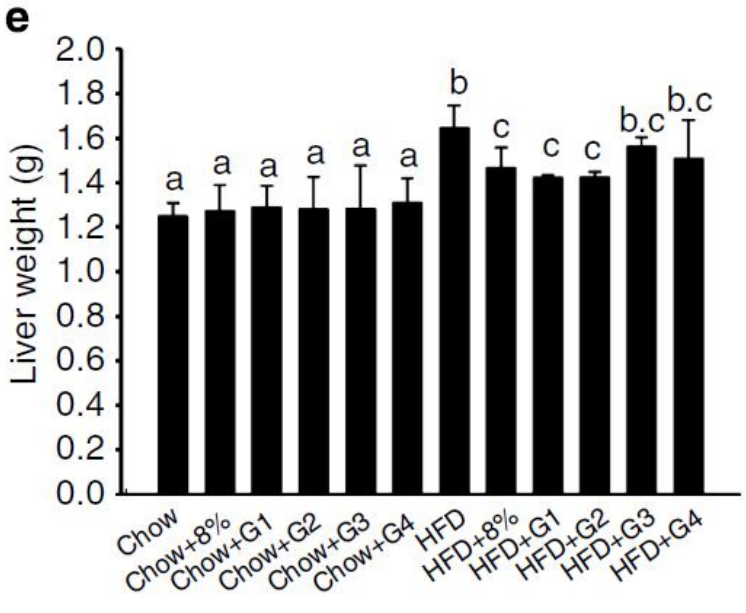
有效多糖成分？



皮下脂肪



附睾脂肪



肝脏重量

4 讨论

药用真菌

血糖

II 型糖尿病

肠道微生物

炎症

肥胖

大分子多糖

具体细菌功能

probiotic *Bifidobacterium* spp., which were previously reported to reduce obesity[51,52], were not detected in the present study. This observation suggests that WEGL may produce anti-obesity effects by altering the Firmicutes-to-Bacteroidetes ratio as well as modifying the levels of other specific bacterial species (Supplementary Data 2 and 3).

A previous study showed that chitin-glucan fibres modulate *Clostridium* cluster XIVa (*Roseburia* spp.) in the gut microbiota of HFD-fed mice[53]. Bacteria belonging to *Clostridium* clusters XIVa, XVIII and IV, which lack prominent toxins and virulence factors, were found earlier to modulate host fatty acid metabolism, induce Treg cell activity and attenuate colitis[54]. Furthermore, *Eubacterium* spp. induced by prebiotic oligosaccharides produce beneficial effects on animal hosts[55], highlighting the potential probiotic effect of these species. Our results demonstrate that WEGL supplementation enhances bacterial levels of *Clostridium* clusters IV, XVIII and XIVa (*Roseburia* spp.), and *Eubacterium* spp. in HFD-fed obese mice (Fig. 4d,e and Supplementary Data 2). These results indicate that the effects of WEGL may be at least partially due to an increase in the populations of these beneficial species. WEGL feeding also decreased several bacterial species associated with inflammation and obesity. For instance, *E. fergusonii*, which is associated with HFD-induced inflammation[20], was reduced following WEGL treatment (Fig. 4d,e and Supplementary Data 2). *Oscillibacter* spp. were also reported to increase in HFD-fed mice compared with chow-fed mice, and these bacteria showed a negative relationship with the expression of intestinal tight junction proteins[40]. Consistent with these observations, the 8% WEGL treatment reversed the percentage of *Oscillibacter* spp. in the gut microbiota of HFD-fed mice to a percentage similar to that seen in chow-fed mice (Fig. 4d,e and Supplementary Data 1 and 2). In addition, *Mucispirillum* spp. belonging to Deferribacteres, which are known to colonize the mucus layer, increased in HFD-fed mice compared with chow-fed mice[56].

SCFAs

促炎/抗炎细胞因子

细菌发酵多糖等益生元产生（*Bacteroides* spp.）

疑问：试验中，小鼠炎症减轻是由SCFAs影响导致，还是由于WEGL或其它分子物质的影响？

Polysaccharides (>300 kDa)

作用机制？

specific bacterial species

Thanks for your listening !