六味地黄汤活性成分组方 LW-AFC 对高热量饲料诱导小鼠代谢综合征的改善作用

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摘要: 目的 研究中药新药 LW-AFC 对代谢综合征(MS)的作用及其作用机制。**方法** 高热量饲料喂养昆明小鼠 6 周,同时每天 ig 给予二甲双胍(阳性对照药)0.2 g·kg^{-1}和 LW-AFC 0.2,0.8 和 3.2 g·kg^{-1}。实验结束时测量小鼠体质量和摄食量, 检测空腹血清总胆固醇(TC)、低密度脂蛋白胆固醇(LDL-C)、高密度脂蛋白胆固醇(HDL-C)、C 肽、血糖(FBG)和胰岛素(FINS)含量,并计算稳态模型胰岛素抵抗评价指数(HOMA-IR);测定小鼠内脏脂肪质量(VFM)、计算内脏脂肪系数(VFC),并检测血清瘦素、抵抗素、肿瘤坏死因子 α(TNF-α)、白细胞介素 6(IL-6)和下丘脑神经肽 Y(NPY)含量。TC 和 FBG 含量用酶比色法检测, LDL-C 和 HDL-C 含量用清除法检测,FINS 和 NPY 含量用放射免疫分析法检测,C 肽含量用均相酶联免疫法检测,瘦素、抵抗素、TNF-α 和 IL-6 含量用液相芯片(luminex)法检测,并采用伊红染色法观察肝脏病理改变。**结果** 与正常对照组比较,模型组小鼠腹型肥胖相关指标 VFM 和 VFC、脂代谢相关指标 TC 和 LDL-C 和糖代谢相关指标 FBG 增高($P<0.01$),肝细胞呈弥漫性小泡性脂变,日均摄食量增高($P<0.01$),促食欲肽 NPY、抑食欲激素瘦素和抵抗素增高($P<0.05$)。与模型组比较, LW-AFC 0.2,0.8 和3.2 g·kg^{-1}可降低 TC($P<0.05$)和 LDL-C($P<0.01$)、升高 HDL-C($P<0.05$, $P<0.01$)等脂代谢相关指标水平,降低 FBG, HOMA-IR和 C 肽($P<0.05$, $P<0.01$)等糖代谢相关指标的水平,并可减轻肝脏小泡性脂变等病理损害,提示 LW-AFC对模型小鼠糖脂代谢紊乱及肝脏病理损伤具有改善作用。LW-AFC 0.2,0.8 和3.2 g·kg^{-1}可降低小鼠的日均摄食量和促食欲肽 NPY 水平($P<0.05$),但可显著增高抑食欲激素瘦素的水平($P<0.01$),提示 LW-AFC对模型小鼠的食欲具有抑制作用。不同剂量的 LW-AFC 还可降低血清脂肪因子抵抗素和炎症细胞因子 TNF-α 和 IL-6 的水平($P<0.05$, $P<0.01$);变量聚类分析结果表明,炎症因子 IL-6 与脂代谢关系密切,抑食欲激素瘦素与糖代谢关系密切,而抵抗素与炎症及食欲关系密切。**结论** LW-AFC 可调节血脂、降血糖、改善胰岛素敏感性、减轻肝脏病理损伤,从而改善代谢综合征。通过降低促食欲激素、升高抑食欲激素水平而抑制食欲以及降低炎症细胞因子分泌可能是其改善代谢综合征的部分作用机制。

关键词: 代谢综合征; 高热量饲料; 炎症; 抵抗素; 食欲; 六味地黄汤; LW-AFC
中图分类号: R285 **文献标志码:** A **文章编号:** 1000-3002(2013)03-0367-07
DOI: 10.3867/j.issn.1000-3002.2013.03.011

代谢综合征(metabolic syndrome,MS)是全身性代谢紊乱疾病,临床诊断标准包括腹型肥胖、高血糖或糖耐量减低、脂代谢异常、高血压和胰岛素抵抗等[1-3]。MS预后不良将导致2型糖尿病、冠心病和动脉粥样硬化等心血管疾病,严重威胁人类健康,已成为临床及科研领域的研究热点之一。

目前 MS 的临床治疗措施除有效的行为干预如运动和饮食控制外,尚有药物如经典的降糖、降脂和抗高血压药物等,这些药物多数作用单一,针对 MS 这种多指标的临床症候群效果有限,而 2 型糖尿病一线治疗药物二甲双胍因其广泛调节糖脂代谢且不良反应少,在 MS 治疗中成为首选药物。此外,靶向药物如过氧化物酶体增殖物激活受体激动剂[4]、大麻素受体 1 抑制剂[5]、二肽基肽酶Ⅳ抑制剂[6]等治疗 MS 作用明显,但多数组织特异性差,易导致不良反应,如罗格列酮易导致水肿[7]和缺血性心血管疾病[7-8],利莫那班(rimonabant)易导致抑郁症、头

基金项目: 国家科技重大专项(2009ZX09301-002);国家科技重大专项(2011ZX09102-101-07);国家自然科学基金(90709012);国家自然科学基金(30701073);全军医药卫生科研基金项目(2006203005)

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晕和腹泻等[5],限制了靶向药物的应用。中药具有多环节多靶点的作用特点,治疗或早期干预 MS 等多系统症候群具有明显优势,中医药治疗 MS 已成为重要的研究方向[9-10]。多年临床实践认为,补肾复方有降糖[11]、降脂、改善胰岛素抵抗[12]、降压、减肥和改善血液流变学等作用,还可保护内皮细胞、抑制炎症[12]等,提示补肾复方对 MS 有治疗作用。

六味地黄汤(Liuwei Dihuang decoction,LW)是滋补肾阴的经典方剂,自古就有治疗消渴的临床应用,近代临床实践和实验药理学研究均表明其有调节糖脂代谢[13]、治疗 2 型糖尿病[14-15]和治疗代谢性紊乱疾病的作用[13]。LW-AFC 是我室经多年研究,在阐明 LW 主要药效物质基础和作用机制的基础上开发出的以 LW 活性成分群配伍组成的中药新药。前期研究已表明,LW-AFC 能够增强学习记忆[16-17]、调节神经内分泌免疫调节网络[18]和调节生殖内分泌的作用[19]。但 LW-AFC 对 MS 这种全身性代谢紊乱疾病的治疗作用尚不明确。本研究采用高热量饲料诱导小鼠 MS 模型,观察 LW-AFC 对 MS 的治疗作用并探讨其可能的作用机制。

1 材料与方法

1.1 动物、药物、试剂和仪器

SPF 级昆明小鼠,雄性,18 ~ 20 g,由军事医学科学院动物中心提供,动物许可证号:SCXK-(军)-2007-004,12 h/12 h,室温 23 ~ 25℃,相对湿度 50% ~ 70%,自由摄食和饮水。二甲双胍原料药,纯度 99.61%,北京四环制药厂;LW-AFC 为本研究所从 LW 中提取,质量百分比为多糖 13%,糖苷 13% 和寡糖 74%。高热量饲料,质量百分比为标准饲料 41%,熟猪油 25%,蔗糖 24%,蛋黄粉 9% 和食盐 1%,委托军事医学科学院实验动物中心生产。空腹血清葡萄糖(fasting blood glucose,FBG)、总胆固醇(total cholesterol,TC)、低密度脂蛋白胆固醇(low density lipoprotein-cholesterol,LDL-C)和高密度脂蛋白胆固醇(high density lipoprotein-cholesterol,HDL-C)测定试剂盒,北京中生北控有限公司;空腹血清胰岛素(fasting blood insulins,FINS)放射免疫分析试剂盒,北京北方生物技术研究所,委托北京东亚放免所检测;神经肽 Y(neuropeptide Y,NPY)放射免疫分析,委托北京康源瑞得生物科技有限公司检测;C 肽(connecting peptide)均相酶联免疫测定试剂盒 AlphaLISA 510C,PerkinElmer 公司;血清瘦素、抵抗素、肿瘤坏死因子 α(tumor necrosis factor-α,TNF-α)和白细胞介素 6(interleukin 6,IL-6)测定使用小鼠血清脂肪因子液相芯片试剂盒 Luminex MADPK-71K,美国 Millipore 公司。Napco-200 型 37℃恒温箱,美国 Napco 公司;Enspire2300 型多功能酶标仪,美国 PerkinElmer 公司;Luminex 100™ IS 型液相芯片检测仪,美国 Luminex 公司。

1.2 动物分组、模型制备和给药

动物适应性饲养后,按体质量随机分组,对照组给予标准饲料,模型组、二甲双胍组和 LW-AFC 组给予高热量饲料 42 d;实验期间(0 ~ 42 d)对照组及模型组小鼠每天 ig 给予生理盐水,二甲双胍组每天 ig 给予二甲双胍 0.2 $g \cdot kg^{-1}$,LW-AFC 组分别 ig 给予 LW-AFC 0.2,0.8 和 3.2 $g \cdot kg^{-1}$。

实验结束时检测小鼠摄食量、体质量、血清 TC、LDL-C、HDL-C、C 肽、FBG 和 FINS 含量,并计算稳态模型胰岛素抵抗评价指数(homeostasis model assessment of insulin resistance,HOMA-IR),HOMA-IR = FBG × FINS/22.5;同时取腹腔、肾囊及睾丸处脂肪称重即内脏脂肪质量(visceral fat mass,VFM),并计算内脏脂肪系数(visceral fat coefficient,VFC),VFC(%) = VFM/体质量 × 100%,检测血清瘦素、抵抗素、TNF-α 和IL-6含量,检测下丘脑 NPY 含量(组织匀浆 NPY·g^{-1}/组织蛋白),做肝脏病理切片进行 HE 染色初步观察肝脏病理改变。

1.3 相关血清生化指标的测定

FBG 和 TC 采用氧化酶比色法检测,LDL-C 和 HDL-C 采用清除法检测[20],FINS 和 NPY 采用放射免疫分析法检测,C 肽采用均相酶联免疫法检测,瘦素、抵抗素、TNF-α 和 IL-6 采用液相芯片法检测。

1.4 统计学分析

实验结果数据以 $\bar{x} \pm s$ 表示,采用 GraphPad Prism 5(version 5.01,GraphPad Inc.,USA)进行 ANOVA 方差分析,组间两两比较采用 Student Newman Keuls 检验;多元统计分析采用 SAS 9.1 进行变量聚类分析(Proc Varclus)。

2 结果

2.1 LW-AFC 改善糖脂代谢紊乱

与正常对照组比较,模型组小鼠体质量无明显差异,VFM 和 VFC 显著增高($P<0.01$)(表 1),TC 和 LDL-C 增高($P<0.01$),HDL-C 无明显差异(表 2),FBG 增高($P<0.01$),FINS 无变化,HOMA-IR和 C 肽含量无明显变化(表 3),表明高热量饲料喂养使小鼠形成腹型肥胖、脂代谢紊乱、高血糖及有胰岛素敏感性降低的趋势。与模型组比较,LW-AFC 0.2,0.8和3.2 $g \cdot kg^{-1}$ 组小鼠体质量、VFM、VFC

Tab. 1 Effect of LW-AFC on obesity formation in mice induced by high energy diet

Group	Body mass/g	VFM/g	VFC/%
Normal control	37 ±5	0.9 ±0.5	2.0 ±0.9
Model	36 ±5	2.0 ±0.8 **	4.4 ±1.5 **
Metformin 0.2	34 ±5	1.8 ±0.5	4.3 ±1.1
LW-AFC 0.2	36 ±5	2.0 ±1.0	4.3 ±1.9
0.8	38 ±6	2.5 ±0.9	5.3 ±2.0
3.2	37 ±6	1.8 ±0.6	4.0 ±1.3

Metabolic syndrome mouse model was established by given high energy diet for 6 weeks. While metformin 0.2 g·kg^{-1} and LW-AFC 0.2, 0.8 and 3.2 g·kg^{-1} were ig administered once daily. Water was used instead of drugs in normal and model groups. The body mass was measured when the experiment was end. Coel-fat and orchio-fat were collected and weighted as visceral fat mass (VFM) to calculate the visceral fat coefficient (VFC). VFC (%) = VFM(g) /body mass(g) ×100%. $\bar{x} \pm s$, $n = 10$. ** $P < 0.01$, compared with normal control group.

Tab. 2 Effect of LW-AFC on lipid metabolism in mice induced by high energy diet

Group	TC/ mmol·L^{-1}	LDL-C/ mmol·L^{-1}	HDL-C/ mmol·L^{-1}
Normal control	3.0 ±0.4	0.52 ±0.09	2.87 ±0.33
Model	5.4 ±0.5 **	2.00 ±0.39 **	2.73 ±0.39
Metformin 0.2	4.2 ±0.6 ##	0.92 ±0.27 ##	3.36 ±0.27 ##
LW-AFC 0.2	4.8 ±0.6 #	1.36 ±0.45 ##	3.41 ±0.24 ##
0.8	4.7 ±0.6 #	1.19 ±0.21 ##	3.27 ±0.18 ##
3.2	4.7 ±0.7 #	1.12 ±0.24 ##	3.15 ±0.27 #

See Tab. 1 for the mouse treament. The serum total cholesterol (TC) was measured using oxidase method. Low density lipoprotein-cholesterol (LDL-C) and high density lipoprotein-cholesterol (HDL-C) were detected by clearance method. $\bar{x} \pm s$, $n = 10$. ** $P < 0.01$, compared with normal control group; # $P < 0.05$, ## $P < 0.01$, compared with model group.

和 FINS 无变化,但 FBG($P < 0.05$, $P < 0.01$), TC($P < 0.05$)和 LDL-C($P < 0.01$)降低,HDL-C ($P < 0.05$, $P < 0.01$)增高,且 LW-AFC 0.8 和 3.2 g·kg^{-1}组小鼠 HOMA-IR ($P < 0.05$)降低, LW-AFC 0.8 g·kg^{-1}组小鼠 C 肽($P < 0.05$)降低,表明 LW-AFC 无减轻肥胖的作用,但能够从降血糖、调血脂、减少胰岛素分泌和改善胰岛素敏感性方面治疗 MS。LW-AFC 调节糖脂代谢紊乱的作用与二甲双胍一致,且LW-AFC有明显降低 C 肽的作用。

2.2 LW-AFC 减轻肝病理变化

正常对照组小鼠病理切片显示,正常肝细胞以中央静脉为中心呈放射状排列(图 1A)。与正常对照组比较,模型组小鼠肝轻度脂肪变性,肝细胞呈现弥漫性小泡性脂变(图 1B)。与模型组比较,二甲双胍(图 1C)及 LW-AFC 0.2 g·kg^{-1}(图 1D)组小泡性脂变减轻, LW-AFC 0.8 和 3.2 g·kg^{-1} (图 1E 和 F)组小泡性脂变改善明显,肝细胞恢复放射状排列,表明 LW-AFC 对高热量诱导的肝脏病理损害有改善作用。

2.3 LW-AFC 的作用机制

2.3.1 LW-AFC 抑制食欲

由表4 可看出,与正常对照组比较,模型组小鼠日均摄食量($P < 0.01$)和下丘脑促食欲肽 NPY ($P < 0.05$)明显增高,抑食欲激素瘦素代偿性增高 ($P < 0.05$)。与模型组比较,二甲双胍和 LW-AFC 0.2,0.8 及 3.2 g·kg^{-1}组小鼠日均摄食量降低 ($P < 0.05$);此外,LW-AFC 0.2 和 3.2 g·kg^{-1}组小鼠促食欲肽 NPY 降低($P < 0.05$), LW-AFC 3.2 g·kg^{-1}组小鼠抑食欲激素瘦素明显增高($P < 0.01$),表明 LW-AFC 对高热量诱导的 MS 小鼠模型具有明显抑制食欲的作用,减少能量摄入是其改善 MS 的可能机制。

Tab. 3 Effect of LW-AFC on glycometabolism and insulin resistance in mice induced by high energy diet

Group	FBG/ mmol·L^{-1}	FINS/ pg·L^{-1}	HOMA-IR	C peptide/ ng·L^{-1}
Normal control	4.9 ±0.8	1.21 ±0.22	6.4 ±3.3	60 ±24
Model	7.7 ±1.6 **	1.16 ±0.19	8.3 ±2.5	76 ±48
Metformin 0.2	5.9 ±1.3 #	1.33 ±0.22	7.7 ±2.4	53 ±21
LW-AFC 0.2	5.8 ±2.0 #	1.15 ±0.30	6.4 ±2.5	69 ±37
0.8	4.9 ±2.1 ##	1.11 ±0.12	5.1 ±2.5 #	38 ±18 #
3.2	5.5 ±1.0 #	1.03 ±0.15	5.7 ±1.6 #	44 ±14

See Tab. 1 for the mouse treament. The serum fasting blood glucose (FBG) was measured using oxidase method, fasting blood insulins (FINS) was determined by radioimmunoassay, and C peptide by homogeneous enzyme immunoassay. Homeostasis model assessment of insulin resistance (HOMA-IR) = FBG × FINS /22.5. $\bar{x} \pm s$, $n = 10$. ** $P < 0.01$, compared with normal control group; # $P < 0.05$, ## $P < 0.01$, compared with model group.

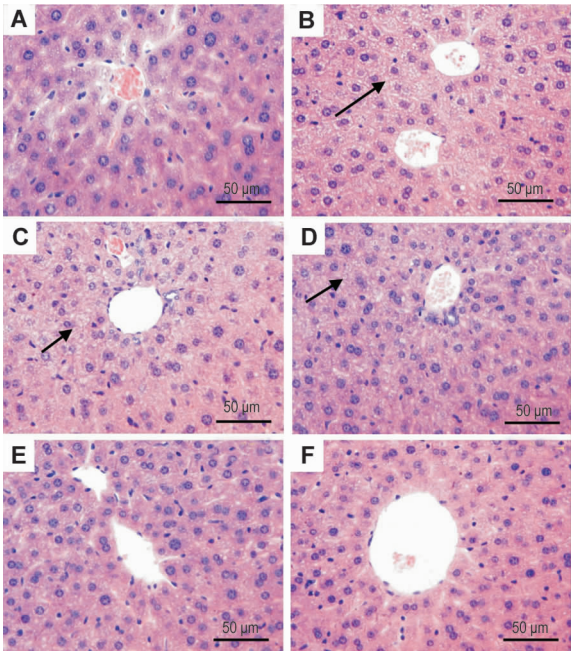

Fig. 1　LW-AFC improved hepatic steatosis in mice induced by high energy diet (HE). See Tab. 1 for the mouse treament. A: normal control; B: model; C: metformin 0.2 g·kg^{-1}; D – F: LW-AFC 0.2, 0.8 and 3.2 g·kg^{-1}, respectively. Arrows showed hepatic steatosis with scattered lipid droplets.

Tab. 4　Effect of LW-AFC on appetite in mice induced by high energy diet

Group	Food consumption/ g	NPY/ μg·g^{-1} protein	Leptin/ μg·L^{-1}
Normal control	5.3 ± 0.7	0.13 ± 0.01	2.8 ± 1.1
Model	6.1 ± 1.1 **	0.23 ± 0.07 *	12.1 ± 6.5 *
Metformin 0.2	4.4 ± 0.9 $^{\#\#}$	0.19 ± 0.04	17.6 ± 7.5
LW-AFC 0.2	5.3 ± 1.3 $^{\#}$	0.16 ± 0.07 $^{\#}$	11.6 ± 5.0
0.8	5.4 ± 1.5 $^{\#}$	0.20 ± 0.05	16.9 ± 10.9
3.2	5.3 ± 1.5 $^{\#}$	0.15 ± 0.03 $^{\#}$	24.8 ± 7.5 $^{\#\#}$

See Tab. 1 for the mouse treament. The food consumption was the average daily intake per mouse. Neuropeptide Y (NPY) was determined by radioimmunoassay, and leptin was measured using liquid chip assay. $\bar{x} \pm s$, $n = 10$. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with normal control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared with model group.

2.3.2　LW-AFC 抑制抵抗素和炎症因子

由表5可看出，与正常对照组比较，模型组小鼠抵抗素增高（$P < 0.05$），炎症因子 TNF-α 和 IL-6 有增高趋势但无统计学意义。与模型组比较，LW-AFC 3.2 g·kg^{-1} 组小鼠抵抗素降低（$P < 0.05$），LW-AFC 0.2，0.8 和 3.2 g·kg^{-1} 组小鼠 TNF-α 降低（$P < 0.05$），LW-AFC 0.8 和 3.2 g·kg^{-1} 组小鼠 IL-6 降低（$P < 0.05$，$P < 0.01$），二甲双胍有降低 TNF-α 和 IL-6 的趋势但无统计学意义。上述结果表明，LW-AFC有抑制抵抗素和炎症的作用。

Tab. 5　Effect of LW-AFC on adipokines in mice induced by high energy diet

Group	Resistin/ μg·L^{-1}	TNF-α/ ng·L^{-1}	IL-6/ ng·L^{-1}
Normal control	6.3 ± 1.4	35.7 ± 7.1	60.5 ± 18.6
Model	8.7 ± 2.5 *	39.3 ± 5.6	71.7 ± 27.7
Metformin 0.2	11.0 ± 2.2	36.7 ± 6.3	49.6 ± 20.3
LW-AFC 0.2	7.6 ± 2.4	30.9 ± 5.2 $^{\#}$	50.6 ± 19.7
0.8	7.8 ± 3.6	29.9 ± 4.7 $^{\#}$	39.7 ± 8.7 $^{\#}$
3.2	6.5 ± 2.3 $^{\#}$	29.7 ± 4.9 $^{\#}$	35.5 ± 2.1 $^{\#\#}$

See Tab. 1 for the mouse treament. The resistin, tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) was measured using liquid chip assay. $\bar{x} \pm s$, $n = 10$. $^{*}P < 0.05$, compared with normal control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared with model group.

2.3.3　变量聚类分析

近年来认为脂肪组织是内分泌器官，肥胖状态下食欲激素紊乱、脂肪组织分泌大量炎症因子与胰岛素抵抗关系密切。对本研究中的糖脂代谢指标、肥胖指标、食欲激素和炎症因子共 13 个变量进行变量聚类法分析，结果表明，13 个指标被聚为 3 类（表6）。炎症细胞因子IL-6与脂代谢指标TC，

Tab. 6　Variables cluster analysis of metabolic syndrome model mice induced by high energy diet

Cluster	Variable	R^2_{Own}	$R^2_{Nearest}$	$(1 - R^2_{Own})/(1 - R^2_{Nearest})$
Cluster 1	IL-6	0.8420	0.3242	0.2337
	TC	0.8735	0.1646	0.1514
	LDL-C	0.7761	0.1167	0.2535
	HDL-C	0.6702	0.1750	0.3997
	FINS	0.4006	0.0546	0.6340
	VFC	0.7471	0.5755	0.5958
Cluster 2	Leptin	0.7196	0.2876	0.3937
	HOMA-IR	0.7066	0.2127	0.3726
	FBG	0.8324	0.1349	0.1938
	C peptide	0.7681	0.1743	0.2808
Cluster 3	Resistin	0.7891	0.0571	0.2237
	NPY	0.5696	0.0869	0.4713
	TNF-α	0.7646	0.0802	0.2560

See Tab. 1 for mouse treament. The R^2_{Own} demonstrated the R^2 of the variable when regressed on the remaining variables in the cluster to which it is assigned. The $R^2_{Nearest}$ is the greatest R^2 when the variable is regressed on any other cluster produced in the analysis. The $(1 - R^2_{Own})/(1 - R^2_{Nearest})$ is a measure of cluster "quality". When a variable has a high R^2 within its own cluster and low to any other, the variable demonstrates a strong fit to the cluster in which it is assigned. $n = 60$.

LDL-C, HDL-C, FINS 和 VFC 共 6 个指标聚成一类,提示炎症因子 IL-6 与脂代谢关系密切;抑食欲激素瘦素与糖代谢指标 HOMA-IR、FBG 和 C 肽共 4 个指标聚成一类,提示抑食欲激素瘦素与糖代谢关系密切;抵抗素与中枢促食欲激素 NPY 及炎症细胞因子 TNF-α 共 3 个指标聚成一类,提示抵抗素与食欲及炎症关系密切。

3 讨论

20 世纪 90 年代随着工业化社会的发展,人类营养摄入增加[21]以及久坐的生活习惯[22]使 MS 发展迅速,成为医学领域关注的热点。本研究采用高热量饲料喂养型 MS 小鼠模型,形成腹型肥胖、血糖增高、血脂紊乱及胰岛素抵抗,符合 MS 诊断标准。本研究结果表明,与二甲双胍一致,LW-AFC 能够改善糖脂代谢紊乱,增加胰岛素敏感性,减轻肝脏病理损伤,表明 LW-AFC 可有效治疗 MS。而且 LW-AFC 还有降低 C 肽含量的作用,C 肽是胰岛素原剪切为胰岛素的另一片段,不受肝脏酶灭活,半衰期比胰岛素长,被认为是评价活性胰岛素即反映 β 细胞合成与释放胰岛素功能的指标。LW-AFC 明显改善血清 C 肽病理性增高的作用提示其有改善高胰岛素血症的潜力。在使用喂养型 MS 小鼠模型初步评价药物对 MS 的作用之后,本课题组还采用了遗传型 *ob/ob* 小鼠模型再评价 LW-AFC 药效。结果发现,与对照组 C57BL/6J 小鼠比较,*ob/ob* 小鼠胰岛素水平由(1.4 ± 0.3)增高至(6.8 ± 2.4)pg·L^{-1}, HOMA-IR 由 6.0 ±3.2 增高至 74.6 ±6.6。与模型组小鼠比较,LW-AFC 给药后上述指标均有明显改变,进一步明确了 LW-AFC 具有改善高胰岛素血症和胰岛素抵抗的作用(待发表)。

据报道,药物可以通过减轻肥胖[23]和抗氧化应激[24]等治疗 MS。对于营养摄入过多导致的 MS 药物能否从能量摄入方面进行干预? 本研究对此进行了初步研究。NPY 是广泛分布于中枢和外周神经系统并维持内环境稳态的激素,参与调节饱腹感、情绪状态、血管张力、胃肠激素分泌以及血管、心肌和脂肪细胞的增殖,在中枢 NPY 能够促进食欲,因此成为节食药物的靶点[25]。瘦素是由脂肪细胞分泌的抑食欲激素,参与调节糖脂代谢平衡和胰岛素敏感性,有抗肥胖作用,而肥胖又会导致瘦素增加即发生瘦素抵抗,与内质网应激有关[26]。与文献报道一致,聚类分析结果表明,抑食欲激素瘦素与胰岛素抵抗和糖代谢关系密切。本研究结果表明,LW-AFC有降低小鼠摄食量、降低 NPY 含量和增加瘦素的作用,表明能够降低食欲、从能量摄入源头减轻代谢负荷过重是 LW-AFC 改善 MS 的作用机制之一。

机体应对能量摄入过多的措施除代偿性地增加循环内瘦素水平以抑制摄食量外,还会促进脂肪细胞的增殖分化以储存过多的能量,但脂肪蓄积将导致脂肪因子和炎症因子分泌增加,继发胰岛素抵抗、血糖增高、脂代谢紊乱和肝脏脂肪变性[27]。文献报道,炎症状态时 TNF-α 和 IL-6 水平增高,将加重胰岛素敏感性下降[28-29]、胰岛细胞凋亡[28]以及肝脏细胞信号因子转导抑制体及脂肪酸合成关键调节因子固醇调节元件结合蛋白 1 过表达而促进肝脏脂肪变性[30]。与文献报道一致,本研究聚类分析结果表明,炎症因子 IL-6 与脂代谢关系密切,而 LW-AFC 能够明显降低炎症因子水平,是其改善 MS 的作用机制之一。

抵抗素是由脂肪细胞产生和分泌的脂肪因子,作用于骨骼肌细胞、肝细胞和脂肪细胞,降低其对胰岛素的敏感性,抵抗素可能是肥胖和胰岛素抵抗之间的重要链接,可直接或间接导致胰岛素抵抗或 MS。胰岛素抵抗或 MS 患者血清抵抗素含量增高,与甘油三酯、胆固醇和极低密度脂蛋白胆固醇含量正相关,与 HDL-C 含量负相关[31]。文献报道,抵抗素通过 NF-κB 途径促进炎症因子 TNF-α 表达,促进肝脏脂肪变性[32],抵抗素增高也可导致下丘脑促食欲肽 NPY 表达增加[33]。与文献报道一致,本研究聚类分析结果表明,抵抗素与炎症因子和食欲激素关系密切,表明抑制抵抗素、抑制与抵抗素关系密切的食欲和炎症可能是 LW-AFC 改善代谢综合征的作用机制。

MS 发病率逐年升高,尤其是肥胖儿童数量增加[34],迫切要求能够长期安全使用、有效干预代谢紊乱疾病的药物出现。LW-AFC 来源于六味地黄汤,该方源自宋代儿科专著《小儿药证直诀》,已经过临床实践证明其安全性。本研究结果表明,LW-AFC能够有效治疗 MS,表现为能够降血糖、调血脂和改善脂肪肝,且在改善胰岛素抵抗和高胰岛素血症、抑制食欲和炎症及调节脂肪因子等内分泌紊乱方面具有应用潜力。

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Improvement effect of LW-AFC, an active component of prescription from Liuwei Dihuang decoction, on metabolic syndrome induced by high energy diet in mice

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Abstract: **OBJECTIVE** To evaluate the effect and mechanism of LW-AFC, an active component from Liuwei Dihuang decoction, against metabolic syndrome (MS) in a high-energy-diet induced model mice with similar characters of human MS. **METHODS** The models of high-caloric-diet-induced Kunming mice were ig given with metformin 0.2 $g\cdot kg^{-1}$ or LW-AFC 0.2, 0.8 and 3.2 $g\cdot kg^{-1}$ consecutively for 6 weeks. At the end of the expreiment, the food intake and body mass of mice were dynamically weighted, and the fast blood total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C), connecting peptide, glucose (FBG), and fasting blood insulins (FINS) were measured. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated after that. The mice were sacrificed, and the coel-fat and orchio-fat were collected and weighted as visceral fat mass (VFM), and the visceral fat coefficient (VFC) was calculated. The levels of serum leptin, resistin, tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) (Luminex method), TC and FBG (oxidase method), LDL-C and HDL-C (clearance method), FINS and NPY (radioimmunoassay) were also detected. Histological micrographs of liver were stained by hematoxylin-eosin. **RESULTS** Compared with normal control group, TC, LDL-C, FBG, HOMA-IR and connecting peptide significantly decreased ($P<0.01$) in diet induced mouse MS model. LW-AFC improved hepatic steatosis, decreased food intake ($P<0.05$), NPY ($P<0.05$), resistin ($P<0.05$), TNF-α ($P<0.05$) and IL-6 ($P<0.05$, $P<0.01$), and increased leptin levels ($P<0.01$), though it had no effect on abdominal obesity and serum insulin levels. The variable cluster analysis showed that IL-6 level was close to lipid metabolism, the level of leptin was close to glucose metabolism, and resistin level was close to appetite and inflammation. **CONCLUSION** LW-AFC might improve MS by reducing hyperglycemia, improving dyslipidemia, increasing insulin sensitivity and reducing the pathologic damage of fatty liver. The possible mechanism might be partly related to its suppressing the appetite and decreasing the levels of inflammatory cytokines.

Key words: metabolic syndrome; high energy diet; inflammation; resistin; appetite; Liuwei Dihuang decoction; LW-AFC

Foundation item: The project supported by National Science and Technology Major Project (2009ZX09301-002); National Science and Technology Major Project (2011ZX09102-101-07); National Natural Science Foundation of China (90709012); National Natural Science Foundation of China (30701073); and Military Medical Research Fund Project (2006203005)

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(收稿日期: 2012-12-30 接受日期: 2013-05-04)
(本文编辑: 齐春会)