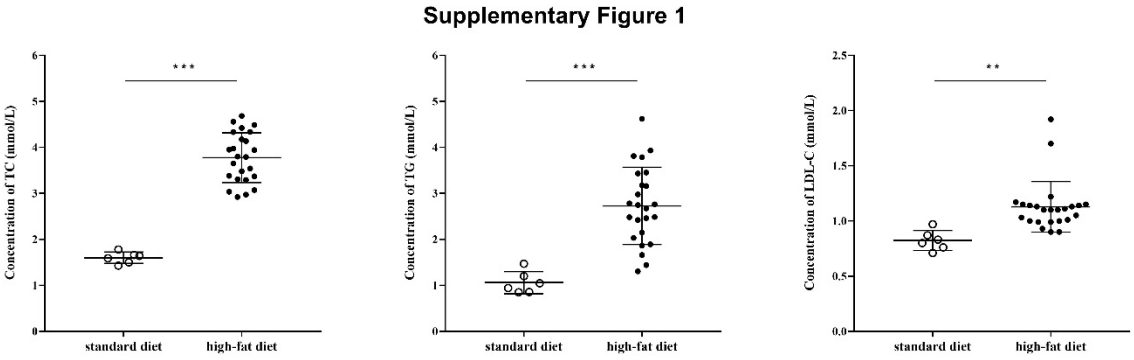
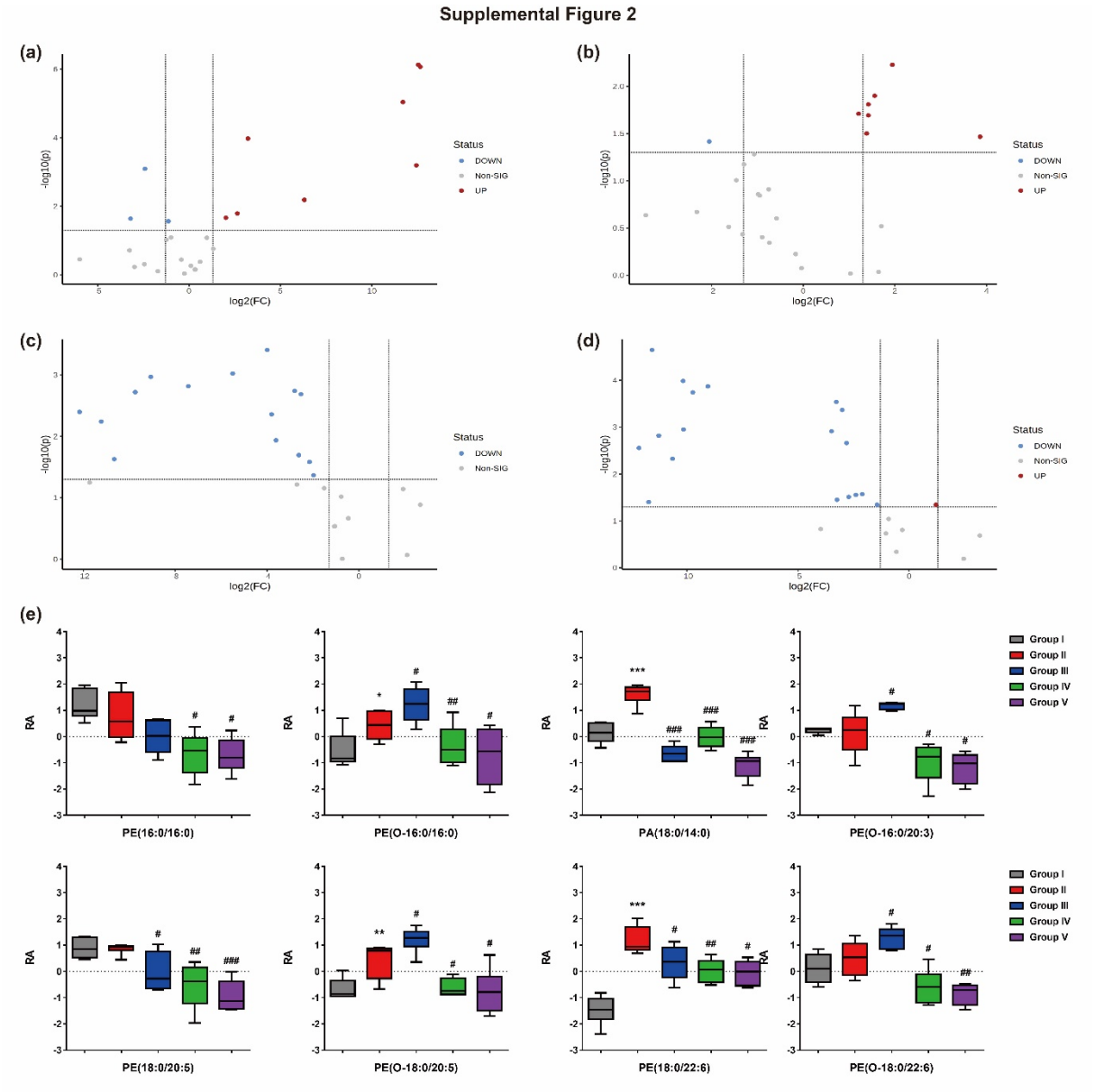
Supplementary data

1. Hyperlipidaemic modeling



Supplementary Figure 1. The serum levels of total triglycerides (TG), total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) in rats after two weeks of normal diet and high-fat diet. n=6 in normal diet and n=24 in high-fat diet. Data are presented as mean ± SD. *t*-test statistics between the two groups, $^{**}p < 0.01$, $^{***}p < 0.001$.

2. Statin-induced oxidative stress

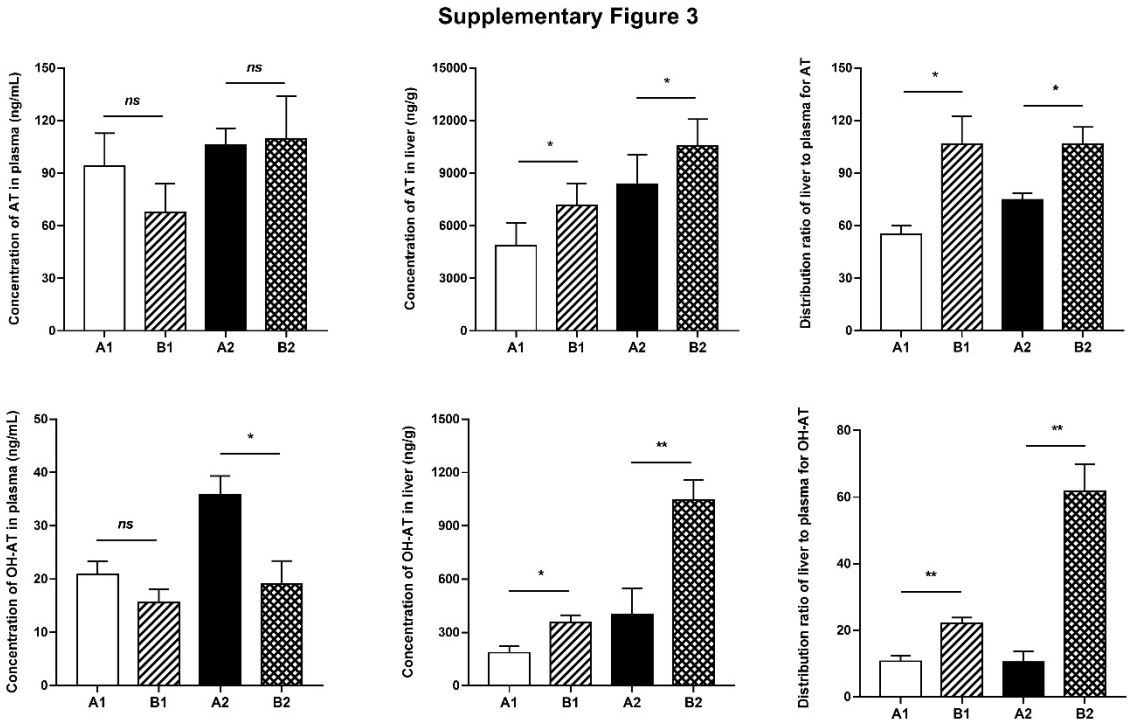


Supplementary Figure 2. Effects of *Ginkgo biloba* extract (GBE) and atorvastatin (AT) on 26 oxidized phosphatidylethanolamines (ox-PEs) in the liver of rats fed a high-fat diet. (a) / (b) / (c) / (d) Volcano plot of multivariate analysis for five test groups, Group II vs Group I, Group III vs Group II, Group IV vs Group II and Group V vs Group II, respectively. red dots / blue dots / gray dots in volcano plot represent up-regulated (1.5 times with significant difference), down-regulated (0.67 times with significant difference) and unchanged lipids, respectively. (e)

Relative abundance (RA) of typical non-oxidized and oxidized phosphatidylethanolamines in the liver of rats in five test groups. Data are presented as mean ± SD. Use *t*-test statistics between Group II and Group I, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. Use one-way ANOVA analysis for data comparison vs Group II (model group), $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$. Group I were normal rats. Group II were hyperlipidaemic rats. Group III were hyperlipidaemic rats given GBE therapy alone. Group IV were hyperlipidaemic rats given AT therapy alone. Group V were hyperlipidaemic rats given GBE and AT.

3. The effect of GBE on the liver exposure of statins

According to the pharmacokinetics study, at about 0.5 h after oral administration of atorvastatin, the plasma concentration of atorvastatin prototype reached its peak in rats. In our pharmacokinetic study, we designed a companion subgroup with six animals for Group A1, Group A2, Group B1 and Group B2. After 0.5 h of the last dose of AT, all rats in the companion subgroups were sacrificed, and liver tissue was immediately collected. The test liver tissue was homogenized with ultrapure water to make a tissue homogenate at 0.1 g/ml. Then, 50 μL of the liver homogenate was mixed with 250 μL of acetonitrile containing tamsulosin (2 ng/mL) to precipitate proteins. Vortex for 5 min, then centrifuge at 30,000 × g for 10 min. Finally, 5 μL of the supernatant was taken to quantitatively analyze the concentrations of AT and OH-AT in the liver sample.



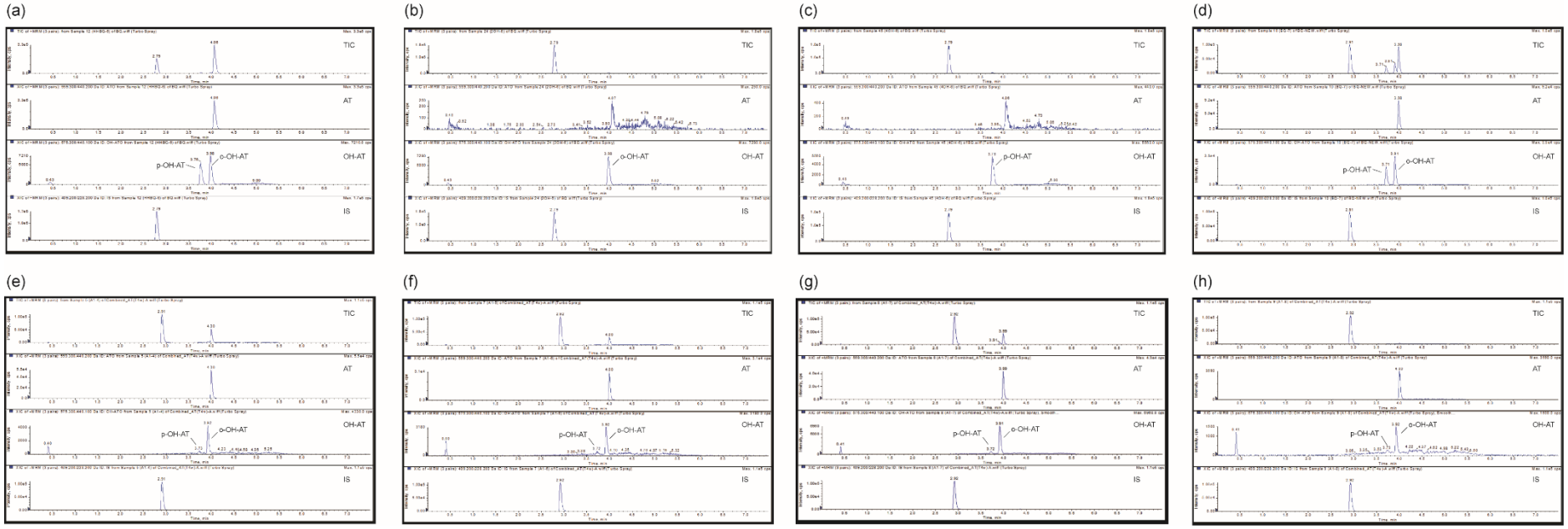
Supplementary Figure 3. The concentrations of atorvastatin (AT) and its ortho-hydroxy metabolite (OH-AT) in rat plasma and liver at 0.5 h after oral administration of AT. Normal

rats in Group A1 were given a single dose of 100 mg/kg ginkgo biloba extract (GBE) and 2 mg/kg AT simultaneously. Normal rats in Group A2 were continuously administered 100 mg/kg GBE once a day for two weeks before a single dose of 2 mg/kg AT. Hyperlipidaemic rats in Group B were administered a single dose of 2 mg/kg AT. Hyperlipidaemic rats in Group B1 were given a single dose of 100 mg/kg GBE and 2 mg/kg AT simultaneously. Hyperlipidaemic rats in Group B2 were administered 100 mg/kg GBE continuously once a day for four weeks before a single dose of 2 mg/kg AT. n=6 of each group. Data are presented as mean ± SD. The ratio of liver concentration to plasma concentration was calculated to obtain the distribution ratio of liver to plasma. Use *t*-test statistics between two groups, 'ns' $p > 0.05$, $^{*}p < 0.05$, $^{**}p < 0.01$.

4. The determination of para-hydroxy atorvastatin in rat plasma

Under our established LC-MS/MS method in the paper, atorvastatin prototype and two hydroxylation metabolites (ortho-hydroxy atorvastatin and para-hydroxy atorvastatin) can be simultaneously measured. Although the MS/MS parameters of the two hydroxylation metabolites were completely identical, complete chromatographic separation of the two metabolites could be achieved under the established chromatographic conditions. Retention times of atorvastatin, para-hydroxy atorvastatin, ortho-hydroxy atorvastatin and IS were 3.98, 3.71, 3.91 and 2.92 min, respectively. As shown in Supplementary Figure 4, the detection signal strength of para-hydroxy atorvastatin in rat plasma was very low, much lower than that of ortho-hydroxy atorvastatin. As shown in Supplementary Table 2, in this pharmacokinetic study, the maximum concentration (C_{max}) of para-hydroxy atorvastatin in rat plasma was only about 10% to 27% of that of ortho-hydroxy atorvastatin.

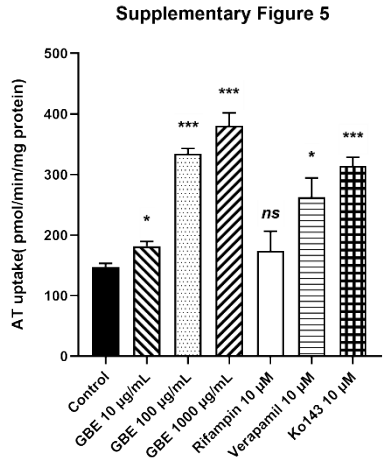
Supplementary Figure 4



Supplementary Figure 4. The typical LC-MS/MS chromatogram of atorvastatin (AT), para-hydroxy atorvastatin (p-OH-AT), ortho-hydroxy atorvastatin (o-OH-AT) and IS. (a) the mixed solution of AT, p-OH-AT, o-OH-AT and IS. (b) the mixed solution of o-OH-AT and IS. (c) the mixed solution of p-OH-AT and IS. (d) the blank rat plasma added with mixed working solution of AT, p-OH-AT, o-OH-AT and IS. (e)/(f)/(g)/(h) the rat plasma samples collected at 30min, 1h, 2h and 4h after atorvastatin administration.

5. The effect of GBE on atorvastatin absorption

Caco-2 cells were seeded into a 24-well plate at a density of 1.0×10^5 cells/well. All cells were first treated with AT at a final concentration of 20 μmol/L. The trial drug was then immediately added, including GBE at a final concentration of 10, 100, 1000 μg/mL, rifampicin at a final concentration of 10 μmol/L, verapamil at a final concentration of 100 μmol/L, Ko143 at a final concentration of 10 μmol/L, and control solvent of 0.1% DMSO. After 10 minutes of incubation, cells were washed by three times, and then cells were lysed by repeated freezing and thawing and homogenization. A Pierce™ BCA Protein Assay kit (Thermo Fisher Scientific, Waltham, MA, USA) was used to determine protein concentrations in cell lysates. Then, 50 μL of the cell homogenate was mixed with 250 μL of acetonitrile containing tamsulosin (2 ng/mL) to precipitate proteins. Vortex for 5 min, then centrifuge at 30,000 × g for 10 min. Finally, 5 μL of the supernatant was taken to quantitatively analyze the concentrations of AT in cell sample.



Supplementary Figure 5. The concentrations of atorvastatin (AT) in Caco-2 cells with different inhibitors, such as ginkgo biloba extract (GBE), rifampin, verapamil and Ko143. n=3 of each group. Data are presented as mean ± SD. Use one-way ANOVA analysis for data comparison vs control, 'ns' $p > 0.05$, $^{*}p < 0.05$, $^{***}p < 0.001$.

6. The effect of GBE on mRNA expression of CYP3A, P-gP and BCRP

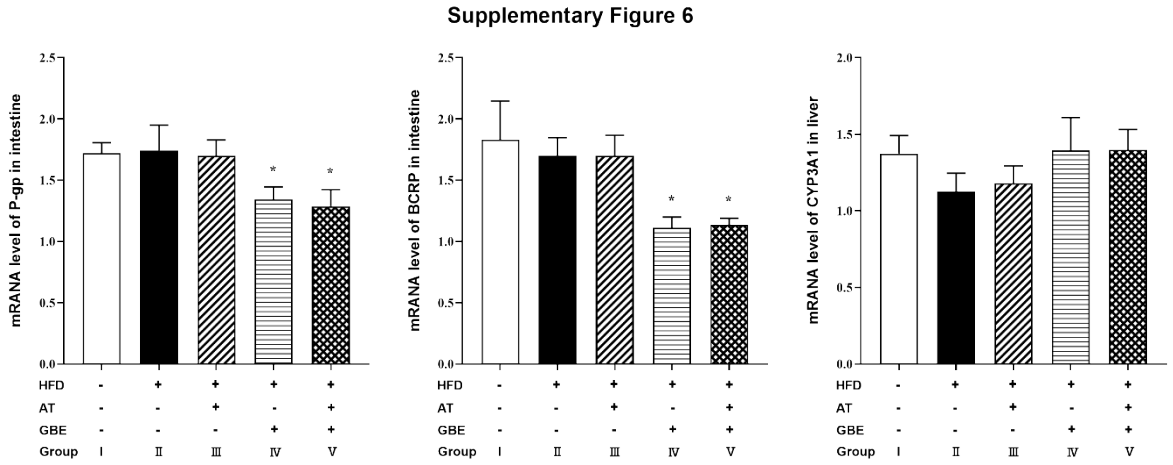
In the pharmacology study, all rats were sacrificed at the end of the experiment to collect the whole liver and other tissues, such as intestine. About 30 mg tissues were used to extracting total RNA according to the manufacturer's instructions using a High Pure RNA Isolation Kit (RNAiso Plus, Takara, Japan), and then reversely converted into cDNA using a PrimeScript™ RT Reagent Kit (Takara Bio, Japan). The qPCR analysis was conducted using SYBR Premix Ex Taq™ (Takara Bio, Japan) and a CFX96 real-time PCR detection system (Bio-Rad, CA, USA). The cycling conditions of the qPCR program were as follows: 95 °C for 10 min, followed by 40 cycles at 95 °C for 10 sec, 55 °C for 1 min, and 72 °C for 30 sec. Three replicates were prepared for each measurement. Melting curve analysis was performed to verify the specificity of the real-time PCR products. The relative quantification was performed using the $2^{-\triangle\triangle}$Ct method, and values were normalized to the reference gene GAPDH. The gene-specific primers of CYP3A1, P-gP and BCRP (see Supplementary Table 1) were synthesized by Invitrogen (Carlsbad, CA, USA).

Supplementary Table 1 Sequences of specific primers for the genes studied in this study.

Gene	Forward Primer (*5'-3' Sequence*)	Reverse Primer (*3'-5' Sequence*)
CYP3A1	ACTGCAGGAGGAGATCGACA	GGTAGTCCCATGAGAATCACCAAA
P-gP	GTGTCACGTGAGGTCGTGAT	AGGGCTGACGGCCAAAATTA
BCRP	GTAGGTCGGTGTGCGAGTCA	AACCAGTTGTGGGCTCATCC
GAPDH	TGTGTCCGTCGTGGATCTGA	TTGCTGTTGAAGTCGCAGGAG

As shown in Supplementary Fig. 6, the gut qPCR results showed that GBE administration did up-regulate the mRNA expression of P-gP and BCRP in rat intestine. However, the liver qPCR results showed that the expression of CYP3A did not change significantly among the

different groups.



Supplementary Fig. 6 The expression levels of CYP3A1 in rat liver and P-gP, BCRP in rat intestine determined using qPCR analysis. Normal rats in Group I were given a regular diet for six weeks. Hyperlipidaemic rats in Group II were given a high-fat diet for six weeks. Hyperlipidaemic rats in Group III and Group IV were continuously administered 2 mg/kg AT or 100 mg/kg GBE once a day from the 3rd to the 6th week of the 6-week high-fat diet. Hyperlipidaemic rats in Group V were administered 100 mg/kg GBE and 2 mg/kg AT at the same time daily from the 3rd to the 6th week. n=6 of each group. Data are presented as mean ± SD. Use one-way ANOVA analysis for data comparison vs Group I (control group), *$p < 0.05$.

7. The metabolic rate of ortho-hydroxylation of atorvastatin

By comparing the $AUC_{0-\infty}$ of ortho-hydroxy atorvastatin and atorvastatin prototype, we could obtain the ortho-hydroxylation metabolic rate of atorvastatin. First, the mass concentration of $AUC_{0-\infty}$ needs to be converted to molar concentration. Second, each animal's metabolic rate must be calculated using its own parameters, not the average. Finally, the results showed that no significant changes were found in the metabolic rate of this pathway in the different test groups (Supplementary Table 2).

Supplementary Table 2 The pharmacokinetics comparison between atorvastatin (AT), ortho-hydroxy atorvastatin (o-OH-AT) and para-hydroxy atorvastatin (p-OH-AT).

Group	C_{max} (ng/mL)			Group	$AUC_{0-\infty}$ (h*ng/mL)		Metabolic rate (%)
	AT	o-OH-AT	p-OH-AT		AT	o-OH-AT	
Group A	55.40 ± 8.27	14.22 ± 3.93	3.78 ± 1.82	Group A	95.42 ± 21.82	50.77 ± 15.55	61.48 ± 15.35
Group A1	108.27 ± 40.81	26.54 ± 5.74	3.93 ± 4.23	Group A1	136.40 ± 21.21	80.57 ± 5.97	61.14 ± 7.45
Group A2	125.80 ± 34.98	38.34 ± 5.90	5.71 ± 0.89	Group A2	145.90 ± 29.25	90.70 ± 10.92	57.32 ± 11.01
Group B	68.48 ± 14.22	16.18 ± 2.28	3.73 ± 2.74	Group B	111.70 ± 40.57	50.17 ± 2.74	55.93 ± 8.56
Group B1	64.27 ± 12.74	20.88 ± 3.22	2.14 ± 0.81	Group B1	108.33 ± 8.00	65.72 ± 9.48	57.27 ± 6.89
Group B2	186.33 ± 74.91	24.16 ± 5.51	5.77 ± 4.22	Group B2	178.33 ± 21.26	66.45 ± 4.32	46.22 ± 10.82
Group C	73.33 ± 27.35	23.17 ± 5.58	5.28 ± 3.36	Group C	125.70 ± 18.69	83.20 ± 18.90	57.61 ± 3.27
Group C1	191.05 ± 61.59	30.28 ± 11.70	6.70 ± 5.64	Group C1	169.80 ± 17.74	108.63 ± 59.09	57.55 ± 6.47

Normal rats in Group A were administered a single dose of 2 mg/kg atorvastatin (AT). Normal rats in Group A1 were given a single dose of 100 mg/kg ginkgo biloba extract (GBE) and 2 mg/kg AT simultaneously. Normal rats in Group A2 were continuously administered 100 mg/kg GBE once a day for two weeks before a single dose of 2 mg/kg AT. Hyperlipidaemic rats in Group B were administered a single dose of 2 mg/kg AT. Hyperlipidaemic rats in Group B1 were given a single dose of 100 mg/kg GBE and 2 mg/kg AT simultaneously. Hyperlipidaemic rats in Group B2 were administered 100 mg/kg GBE continuously once a day for four weeks before a single dose of 2 mg/kg AT. Hyperlipidaemic rats in Group C were continuously administered 2 mg/kg AT once a day for four weeks. Hyperlipidaemic rats in Group C1 were continuously administered 100 mg/kg GBE and 2 mg/kg AT once daily for four weeks. n=6 of each group. Data are presented as mean ± SD. The ortho-hydroxylation metabolic rate of atorvastatin (Metabolic rate) was obtained by comparing the $AUC_{0-\infty}$ of ortho-hydroxy atorvastatin and atorvastatin prototype.

8. The ethical approvement materials for animal research

药学动物实验中心实验动物福利伦理审查申请表

受理编号（由受理部门填写）：CPU-PK-202111-017　申请日期：2021 年 11 月 02 日

一、申请者基本情况			
项目名称	银杏叶提取物联合阿托伐他汀用于高脂血症大鼠的药效学及药代动力学研究		
项目来源	☑ 1.纵向（中央/地方财政资金资助） ☐ 2.横向（社会资金资助）		
实验种类	☐ 1、药物疫苗类　☐ 2、生物类　☐ 3、教学训练类 ☐ 4、健康食品类　☑ 5、医学研究类　☐ 6、其它		
申请部门	药物科学研究院药物代谢动力学重点实验室		
项目负责人	孙金国/彭英	电话： 13915990907/13770828062	E-mail： jgsun@cpu.edu.cn 1020162518@cpu.edu.cn
联系人	王庆庆	电话：19850856036	E-mail： ycbswqq@gmail.com

二、所需实验动物		
来源：上海实验动物研究中心	质量合格证 ☑ 有　☐ 无	
品种/品系 ☑ 大鼠　☐ 小鼠　☐ 裸鼠 ☐ 豚鼠　☐ 兔 其他（请具体说明）：_____	等级 ☐ 普通 ☐ 清洁 ☑ SPF ☐ 无菌	
数量：78 只（♀ 78 ；♂ ）	体重：200-220g	年龄：6-8 周

十三、承诺

项目负责人承诺书

我承诺该申请使用表的内容准确无误。

我同意遵守中华人民共和国国家科学技术委员会制定的《实验动物管理条例》、中华人民共和国科学技术部发布的《关于善待实验动物的指导性意见》、江苏省人民政府发布的《江苏省实验动物管理办法》。

我承诺包括我自己在内的该申请使用表中提及的与实验动物有接触的人员，已经参加了中国药科大学实验动物中心要求的相关培训，掌握了申请使用表中涉及的动物实验方法，都有能力完成动物实验，并且深知使用这些活体动物及动物组织所存在的风险。

我清楚作为该项目的负责人，有责任承诺本课题组所有成员在本研究工作中均会遵循人道主义原则，确保实验动物的福利伦理，并严格遵守中国药科大学实验动物中心的相关规章制度。

项目负责人签字：

日期：2021 年 11 月 2 日