Hepatic drug metabolism in older people with body composition changes

Running Title: Hepatic drug metabolism in older people

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Abstract

Aim: Dosage adjustment is essential in older individuals because they are prone to experience a decline in liver function and changes in body composition. However, quantitative tests or equations for evaluating the activity of hepatic drug metabolism have not yet been clearly established. We examined hepatic drug metabolism activities in older individuals, focusing on changes in body composition parameters.

Methods: Lansoprazole (LPZ) and nifedipine (NIF), substrates of the metabolic enzymes cytochrome P450 (CYP) 2C19 and 3A4, respectively, were selected to study hepatic drug metabolism. Residual samples from blood test for older patients were evaluated to determine drug metabolism. The body composition of relevant patients was determined by analyzing characteristic parameters of skeletal muscle mass index (SMI), handgrip strength (HGS), and hepatic steatosis index (HSI). The differences in hepatic drug metabolism were studied statistically among categories in terms of the cutoff value of these parameters.

Results: Older male patients receiving LPZ and NIF in the low SMI (<7.0 kg/m^2) category showed an 85-90% reduction in respective CYP2C19 and CYP3A4 metabolic activities compared to the normal SMI category. For the female patients, CYP2C19 and CYP3A4 metabolic activities showed no significant correlation with SMI and HGS. Fatty liver disease (HSI$\geq$36) was found to reduce CYP2C19 metabolic activity particularly in older female patients.

Conclusions: Low CYP2C19 metabolic activity was statistically correlated with low SMI in male patients and high HSI in female patients, whereas low CYP3A4 metabolic activity was statistically correlated with low HGS in male patients.

Keywords

hepatic drug metabolism, older patients, sarcopenia, skeletal muscle mass, handgrip strength

Introduction

Older people tend to experience adverse drug effects more frequently than young people do.[1] This is mainly because of polypharmacy and changes in pharmacokinetics (PK) and pharmacodynamics (PD) in the older population due to declining physiological functions.[2-4] Sarcopenia, a symptom of loss of muscle mass and strength due to factors such as aging, disease, malnutrition, and immobility, has been gaining increased attention as one of the causes of increased adverse effects in geriatrics[5]. Older people generally exhibit changes in muscle mass, muscle strength, body fat, body water, and other features related to body composition and sarcopenia because of the aging process[6,7]. Body composition parameters can be predicted to affect PK/PD in older individuals. Consequently, the accurate evaluation of drug metabolism and elimination in the liver and kidneys is particularly relevant to achieve the appropriate drug therapy for older people. However, laboratory tests or test values for the quantitative evaluation of hepatic drug metabolism have not yet been clearly established; hence, a relevant equation similar to that used for evaluating renal drug excretion is not yet available.[8,9]

Cytochrome P450 (CYP) enzymes in the liver and intestine are essential for metabolizing almost two-thirds of drugs, and the CYP1A2, 2C9, 2C19, 2D6, and 3A4 isoforms play a major role.[10,11] To date, a 'cocktail method' has been used to evaluate the hepatic drug metabolism *in vivo*.[12-15] In the cocktail method, a mixture of probe drugs, in which each drug is a substrate for one particular enzyme, is administered and then blood samples are collected and analyzed. Subsequently, the data is used to construct the concentration-time curve of each probe drug and its metabolites to determine the area under the concentration-time curve (AUC). The ratio $AUC_{metabolite}/AUC_{drug}$ is then used to evaluate the hepatic drug metabolism. This method is often used in healthy adults, but

rarely in older people, because it involves administering non-essential drugs and collecting multiple blood samples.[12-14]

In this study, we propose a simple quantitative method for evaluating hepatic drug metabolism that involves using residual blood samples from patients receiving drugs and measuring their body composition parameters. In selecting ‘probe drugs’, we focused on those that are metabolized by a single enzyme to form a single metabolite. We examined changes in the activity of drug metabolizing enzymes and the body composition of older individuals to statistically determine how this relationship affects hepatic drug metabolism.

Methods

Probe drugs

We focused on lansoprazole (LPZ) and nifedipine (NIF), which are substrates of CYP2C19 and 3A4, respectively and measured their plasma concentrations. We also measured plasma concentrations of 5-hydroxy lansoprazole (5OH-LPZ) and dehydronifedipine (DNIF), which are metabolites of LPZ and NIF, respectively (Table S1).

Drug metabolism study

The drug metabolic activity of each enzyme was defined by the ratio of the measured metabolite concentration to the probe drug concentration as follows:

$$\text{Drug metabolic activity} = \frac{\text{metabolite concentration (μM)}}{\text{probe drug concentration (μM)}}.$$

Blood was sampled at several time points to construct a concentration-time curve and the drug metabolic activity was evaluated using the parameter $AUC_{metabolite}/AUC_{probe\ drug}$. Because one blood sampling value was utilized in this study, we employed the parameter $C_{metabolite}/C_{\ probe\ drug}$ based on metabolite and drug concentrations.

Patients

All the data presented in this study were obtained from patients aged ≥ 65 years who were hospitalized at Sunagawa City Medical Center (Hokkaido, Japan) from May to July 2018. The selected patients were daily receiving the probe drugs LPZ and/or NIF orally. Therefore, we anticipated that the steady-state plasma concentration of the drugs would be achieved some time after initiation of administration. We selected patients who were

scheduled to have blood collected as part of their treatment. In almost all patients, blood samples were collected 24 h before dosing, which corresponds to the trough value of the drug concentration-time profile.

All patients provided written informed consent before participating in the study. The body composition of the participants was measured and their residual blood samples were collected after their blood tests. We also collected information on their test results for aspartate aminotransferase (AST), alanine transaminase (ALT), γ-glutamyl transpeptide (γ-GTP), serum albumin, total bilirubin, serum creatinine, and blood urea nitrogen (BUN) levels. Here, the correlation between drug metabolic activity and liver function markers such as AST, ALT, γ-GTP, and total albumin, was obtained to study the effect of changes in the liver. Data on the use of co-administered drugs were also collected.

Body composition measurements and parameters

Body composition was measured using the bioelectrical impedance method using a TANITA InnerScan DUAL RD-800 (TANITA, Tokyo, Japan) in the standing position, and an InBody S10 (BioSpace, Urbandale, IA, USA) in the prone position. Body weight (kg) and body mass index (BMI, kg/m^2) were also determined in addition to the body composition parameters of limb skeletal muscle mass (LSM, kg), body fat (%), body water (%), and bone mineral (kg). The skeletal muscle mass index (SMI, kg/m^2) was obtained by dividing the LSM (kg) by the square of the height (m^2).[16,17] The cutoff values of the SMI were set at 7.0 kg/m^2 and 5.7 kg/m^2 for male and female patients, respectively according to the guidelines of the Asian Working Group for Sarcopenia (AWGS).[16]

Handgrip strength (HGS, kg) was measured using a Smedley-type mechanical handgrip dynamometer (Matsumiya Ika Seiki Seisakujo, Tokyo, Japan). HGS was

measured for the right and left hands, and the higher value was adopted. The cutoff HGS values were set at 28 kg and 18 kg for male and female patients, respectively following the AWGS recommendations.[18,19] We focused on sarcopenia diagnostic and body composition parameters in older people. We determined the metabolism of the LPZ and NIF probe drugs based on these parameters (Fig. S1).

Hepatic steatosis index (HSI) was calculated using the following equation:

$$\mathrm{HSI} = 8 \times \frac{\mathrm{ALT}}{\mathrm{AST}} + \{\mathrm{BMI} + 2[\text{if diabetes}] + 2[\text{if female}]\}.$$

Fatty liver was defined as HSI≥36 for both males and females. [20]

Measurement of plasma concentration of probe drugs and metabolites

Plasma concentrations of the probe drugs and metabolites were measured using ultraperformance liquid chromatography in combination with triple quadrupole mass spectrometry. Details are provided in Doc. S1.

Statistical analysis

Statistical analyses were performed using the statistical package for the social sciences (SPSS) for Windows version 25 (released 2017, IBM Corp., Armonk, NY, USA). The Shapiro-Wilk test was used to evaluate the normality of the distribution of all parameters. The Mann-Whitney U test and Kruskal-Wallis test were used to analyze differences between two categories and among more than three categories, respectively. In the Kruskal-Wallis test, *P*-values were adjusted using the Bonferroni method. Correlation coefficients were tested using the Spearman's rank test.

Ethical guidelines

This study was conducted in compliance with the Declaration of Helsinki and the Ethics Guidelines for Human Clinical Trials. This study was approved by the Ethics Committee of the Faculty of Pharmaceutical Sciences, Hokkaido University (2017-003). The study protocol was approved by the institutional review board of Sunagawa City Medical Center.

Results

The 78 (34 men and 44 women) participants in this study were divided into 46 (17 men and 29 women) who received LPZ (S-LPZ cohort) and 32 (17 men and 15 women) who received NIF (S-NIF cohort). The clinical characteristics of the patients are summarized in Table 1. The results of the blood tests are shown in Table 2.

The effect of SMI on the metabolic activity of CYP2C19 was evaluated in male participants in the S-LPZ cohort using the Mann-Whitney U test. The results showed that the LPZ metabolism level of patients in the SMI<7 category was 0.11 times higher than that of patients in the SMI≥7 category (P=0.079, Fig. 1a). No significant difference in LPZ metabolism was observed between female patients in the SMI<5.7 and SMI≥5.7 categories in the S-LPZ cohort (data not shown).

The effect of HGS on CYP2C19 metabolic activity was evaluated for male patients in the S-LPZ cohort using the Mann-Whitney U test. LPZ metabolism level of patients in the low HGS<28 kg category was 0.082 times higher than that of patients in the normal HGS category ($^{*}P$=0.009 (<0.05), Fig. 1b). The female patients were classified into two categories based on an HGS cutoff value of 18 kg and no significant difference in CYP2C19 metabolic activity was observed between the two categories (data not shown).

Furthermore, the combined effect of SMI and HGS was studied by dividing male patients in the S-LPZ cohort into the following three categories: 1) normal category, normal SMI and normal HGS; 2) moderate category, low SMI or low HGS; and 3) sarcopenia category, low SMI and low HGS. The Kruskal-Wallis test was performed on these three categories to evaluate LPZ metabolism, and levels in the moderate and sarcopenia categories were 0.0027 and 0.082 times higher than those in the normal

category (P=0.071 and P=0.056), respectively.

The result of the Mann-Whitney U test showed that CYP2C19 metabolic activity for the category of HSI≥36 was 0.10 times higher than that for the normal category of HSI<36 (Fig. 2, $^{*}P$=0.017). In particular, for female patients, a strong correlation coefficient of -0.481 ($^{*}P$ =0.008) between CYP2C19 metabolic activity and HSI was observed using the Spearman's rank test.

Similar to the male patients in the S-LPZ cohort, CYP3A4 metabolic activity of male S-NIF patients in the SMI<7 category was 0.15 times higher than that in male patients in the SMI≥7 category ($^{*}P$=0.044, Fig. 3). Furthermore, in the S-NIF cohort, a Spearman's rank correlation coefficient value between CYP3A4 metabolic activity and SMI of male patients was 0.596 ($^{*}P$=0.012). A strong correlation was obtained between CYP3A4 metabolic activity and SMI in the male S-NIF cohort. For male patients in the S-NIF cohort, the Kruskal-Wallis test showed that the CYP3A4 activity of patients in the moderate category was 0.041 times higher than that of patients in the normal category, with less significant P-value of 0.18.

In the S-NIF cohort, there were no significant differences in CYP3A4 metabolic activity between the two categories of HSI≥36 and <36 (data not shown).

Discussion

We studied hepatic drug metabolic activity in older patients, focusing on body composition and sarcopenia parameters of SMI and HGS. First, we evaluated the metabolism of LPZ and NIF in the study population and compared it with that in healthy adults. [21, 22] The data on LPZ and 5OH-LPZ metabolism in healthy adults showed that the $AUC_{5OH\text{-}LPZ}/AUC_{LPZ}$ ratio was 0.081, and the trough value of the blood concentration ratio $C_{5OH\text{-}LPZ}/C_{LPZ}$ was estimated to be 0.37, using the published half-life and peak value of the drug.[21] For LPZ, the blood concentration ratio at the trough was predicted to be approximately 4.6 times higher than the AUC ratio because of the difference in half-life between the drug and metabolite. In contrast, in healthy adults, the trough AUC and blood concentration ratios of NIF and DNIF were 0.34 and 0.30, respectively.[22] Thus, in this case, the trough value of the blood concentration ratio could be used as a surrogate parameter.

In this study, the metabolic activity of CYP2C19 was evaluated using the parameter $C_{5OH\text{-}LPZ}/C_{LPZ}$. The median values of CYP2C19 metabolic activity in male and female patients in the S-LPZ cohort were 0.023 and 0.086, respectively (Table 1). Similarly, the median values of CYP3A4 metabolic activity (C_{DNIF}/C_{NIF}) in the S-NIF cohort were 0.11 and 0.46 in male and female patients, respectively. Therefore, the metabolic activities of CYP2C19 and CYP3A4 likely declined with age in the study population that consisted of older adults and, in particular, in the male patients.

The results of blood sampling (Table 2) indicated that none of the patients in this study exhibited notable abnormalities of liver function markers. In addition, no significant correlation was found in the Spearman's rank test between liver function markers and CYP metabolic activity. Therefore, the effect of liver diseases, which cause abnormalities

of liver function markers, on the decline in hepatic drug metabolism is considered to be minor. In addition, one male patient in the S-NIF cohort was received CYP3A4 inhibitor of verapamil, however, the plasma concentration of NIF did not noticeably increase. Thus, it is unlikely that co-administered drugs had an effect on hepatic drug metabolism (Table S2).

CYP2C19 metabolic activity declined in male patients in the low SMI (<7.0) and low HGS (<28) categories of the S-LPZ cohort (Fig. 1a and b). Therefore, a decline in SMI and HGS, which are indicators of muscle mass and strength, respectively, likely caused the lower CYP2C19 metabolic activity in male patients in the S-LPZ cohort.

Parkinson et al.[23] reported that the CYP2C19 metabolic activity in a cohort aged>60 years declined by at least 25% compared with that of a cohort<20 years old. Overall, CYP2C19 activity in male patients in the S-LPZ cohort was lower than that in healthy adults.

In contrast, CYP2C19 metabolic activity in female patients in the S-LPZ cohort showed no correlation with SMI. Therefore, the effect of SMI on CYP2C19 activity was low in older women who maintain muscle mass. We observed a clear difference in the effect of SMI on CYP2C19 activity between male and female patients. However, Parkinson et al.[23] reported that CYP2C19 activity is likely lower in female patients than it is in male patients. Further studies are needed to clarify the sex differences in the age-related reduction of CYP2C19 activity.

CYP3A4 metabolic activity in male patients in the S-NIF cohort tended to increase with increasing muscle mass, and a strong Spearman's rank correlation coefficient between CYP3A4 metabolic activity and SMI was obtained.

Concerning sex differences observed in hepatic drug metabolism due to the change of

SMI, we studied age dependence of SMI for the present cohorts. SMI was found to be almost constant over the entire range of age ≥65 years for males and females. The values of SMI were generally equally distributed above and below the cutoff value of 7.0 for males, while they were mostly distributed above the cutoff value of 5.7 for females. It is likely that these different distributions of SMI resulted in a sex difference in hepatic drug metabolism (Fig. S2).

In the S-LPZ cohort, CYP2C19 metabolic activity declined significantly in the high HSI (≥36) category ($^{*}P$=0.017, Fig. 2). In particular, female patients in the S-LPZ cohort exhibited a strong negative correlation between HSI and CYP2C19 activity. This indicates that accumulation of fat, especially in the liver, tends to influence the reduction in CYP2C19 activity more significantly than the accumulation of muscle mass in older female patients. Kim et al.[20] reported that HGS was significantly decreased in patients with fatty liver disease. This indicates that fatty liver may be associated with sarcopenia. Considering these results, the present study suggests that the decline in CYP2C19 metabolic activity in older people is likely to be affected by fatty liver.

Although the total CYP content of the liver declines with age,[24] this decrease does not always directly lead to a decline in CYP activity.[24-26] The metabolic activity of CYP2D6 on its substrate dextromethorphan is less likely to be affected by age. [24-25,27-28] In contrast, the enzymatic activity of CYP2C19 on its substrates of LPZ and omeprazole is likely to decline with age, [24-25] and there is a difference of opinion on the effect of aging on CYP3A4 metabolic activity on nifedipine, midazolam, and cyclosporin.[28] Therefore, the activity of enzymes on drugs is not likely predictable based on age alone. In addition, because the present study population included older patients who had certain diseases, other factors such as sarcopenia diagnostic parameters (SMI, HGS) and body composition

parameters (SMI, body fat [%]), need to be considered to account for the reduction in drug metabolism activity. However, drug metabolic processes depend on enzyme subtypes, and changes in enzymatic activity due to aging are not clearly understood. Kim et al.[29] reported that the total decline in muscle mass change with aging is lower in women than it is in men. In addition, Yoon et al.[30] reported that middle-aged and older men with severely low skeletal muscle mass are at high-risk for developing albuminuria. However, additional studies are needed to clarify changes in CYP activity with age and sex.

This study had some limitations. First, we used residual blood samples of patients and each measurement was performed only once. A more accurate evaluation of liver drug metabolism needs to be performed using multiple measurements. However, the method used in the present study could be particularly useful in older patients because it involves the non-invasive evaluation of residual blood samples and body composition parameters.

In conclusion, a reduction in SMI and HGS below the sarcopenia diagnostic criteria was found to be correlated with a decline in CYP2C19 and CYP3A4 metabolic activity in male older patients, whereas, for older female patients, fatty liver disease was shown to influence a decline in CYP2C19 metabolic activity.

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Disclosure statement

The authors declare no conflict of interest.

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Tables

Table 1 Clinical characteristics of patients

Parameter	Probe drugs Lansoprazole (CYP2C19)	 Nifedipine (CYP3A4)
Number of patients	46	32
Female, n (%)	29 (63.0)	15 (46.9)
Age (year)	76.3±7.29	75.28±7.14
Height (m)		
Male	1.64±0.061	1.66±0.061
Female	1.46 [0.055]	1.49±0.050
Body weight (kg)		
Male	59.16±10.49	63.50±7.91
Female	54.39±9.64	53.93±11.38
Body mass index, BMI (kg/m^2)		
Male	21.88±3.40	23.11±2.74
Female	25.19±4.08	24.11±4.51
Muscle mass (kg)		
Male	42.12±6.66	46.02±6.34
Female	32.06±3.08	33.86±3.42
Skeletal muscle mass index, SMI (kg/m^2)		
Male	6.74±1.58	7.52±1.25
Female	6.61±1.09	6.70±1.13
Body fat (%)		
Male	24.09±7.54	23.14±7.03
Female	36.28±8.89	31.52±10.70-
Hand grip strength, HGS (kg)		
Male	24.79±8.26	28.35±5.90
Female	16.61±5.50	17.82±5.02
Metabolite/Drug ratio		
Male	0.023 [0.091]	0.112 [0.73]
Female	0.086 [0.14]	0.46 [1.62]

The data are presented as the mean and standard deviation (mean± SD) for normally distributed variables and as the median (interquartile range) for non-normally distributed variables.

Table 2: Blood sampling test data

Parameter	Probe drugs	
	Lansoprazole (CYP2C19)	Nifedipine (CYP3A4)
AST (IU/L)	18.0 [9.0] (n = 45)	18.0 [7.25] (n = 30)
ALT (IU/L)	12.0 [5.0] (n = 45)	15.5 [11.0] (n = 30)
γ-GTP (IU/L)	24.0 [31.0] (n = 43)	23.5 [40.0] (n = 28)
Albumin (g/dL)	3.50 [0.60] (n = 39)	3.34±0.43 (n = 29)
Total bilirubin (mg/dL)	0.47 [0.26] (n = 42)	0.47 [0.26] (n = 28)
Serum creatinine (mg/dL)		
Males	0.83 [0.56] (n = 16)	1.07 [1.01] (n = 15)
Females	0.81 [0.33] (n = 29)	0.79 [0.70] (n = 15)
BUN (mg/dL)	15.3 [9.08] (n = 44)	18.8 [15.1] (n = 31)

Data are presented as the mean ± standard deviation (SD) for normally distributed variables and the median (interquartile range) for non-normally distributed variables. AST, aspartate aminotransferase; ALT, alanine transaminase; γ-GTP, γ-glutamyl transpeptide; BUN, blood urea nitrogen.

Figure legends

Figure 1 Boxplot of cytochrome P450 2C19 (CYP2C19) metabolic activity comparing male patient bisected categories of (a) skeletal muscle mass index (SMI) with the cutoff value of 7.0 kg/m^2 and (b) handgrip strength (HGS) with the cutoff value of 28 kg; * $P<0.05$ was fulfilled by the Mann-Whitney U test.

Figure 2 Boxplot of cytochrome P450 2C19 (CYP2C19) metabolic activity comparing male and female patient bisected categories of (a) hepatic steatosis index (HSI) with the cutoff value of 36; * $P<0.05$ was fulfilled by the Mann-Whitney U test.

Figure 3 Boxplot of cytochrome P450 3A4 (CYP3A4) metabolic activity comparing male patient bisected categories of skeletal muscle mass index (SMI) with the cutoff value of 7.0 kg/m2; * $P<0.05$ was fulfilled by the Mann-Whitney U test.

Supporting information

Supplementary documents

Doc. S1 Analysis of plasma concentration of probe drugs and metabolites and Chemical reagents

Supplementary Tables

Table S1: Probe drugs

Table S2: Co-administered drugs used

Supplementary figures

Figure S1: Parameters used in this study.

AWGS, Asian working group for sarcopenia.

Figure S2:

(a) Plot of SMI versus age for male patients in S-LPZ cohort.

The best fit (dotted) line of SMI with age and the cutoff value of 7.0 (red line) for males are shown.

(b) Plot of SMI versus age for female patients in S-LPZ cohort.

The best fit (dotted) line of SMI with age and the cutoff value of 5.7 (red line) for females are shown.

(c) Plot of SMI versus age for male patients in S-NIF cohort.

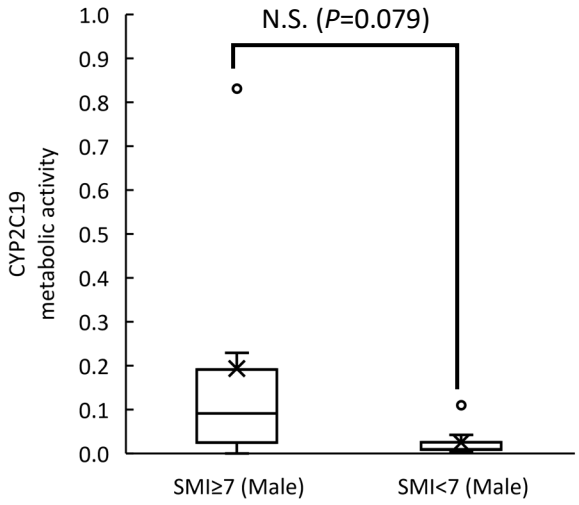
The best fit (dotted) line of SMI with age and the cutoff value of 7.0 (red line) for males are shown.

(d) Plot of SMI versus age for female patients in S-NIF cohort.

The best fit (dotted) line of SMI with age and the cutoff value of 5.7 (red line) for females are shown.

Figure 1

(a)


N.S. (P=0.079)
CYP2C19 metabolic activity
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
SMI≥7 (Male)
SMI<7 (Male)

(b)

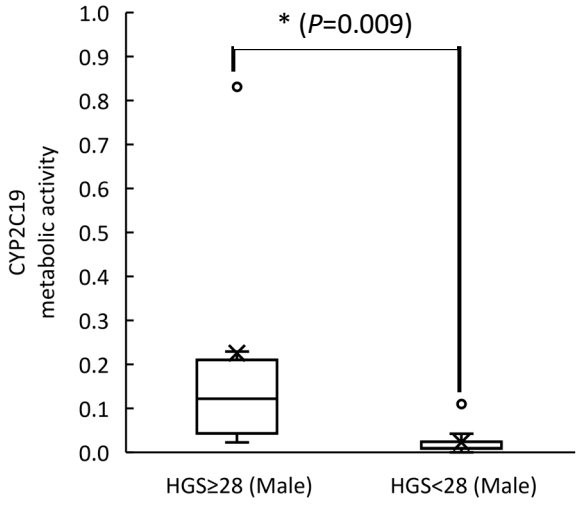

* (P=0.009)
CYP2C19 metabolic activity
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
HGS≥28 (Male)
HGS<28 (Male)

Figure 2

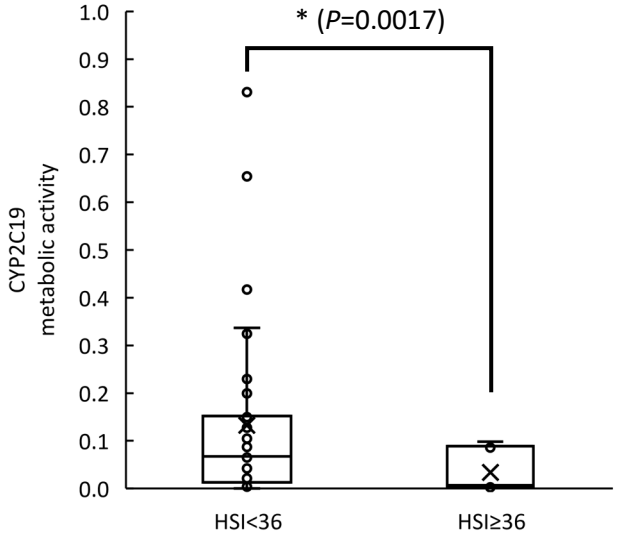
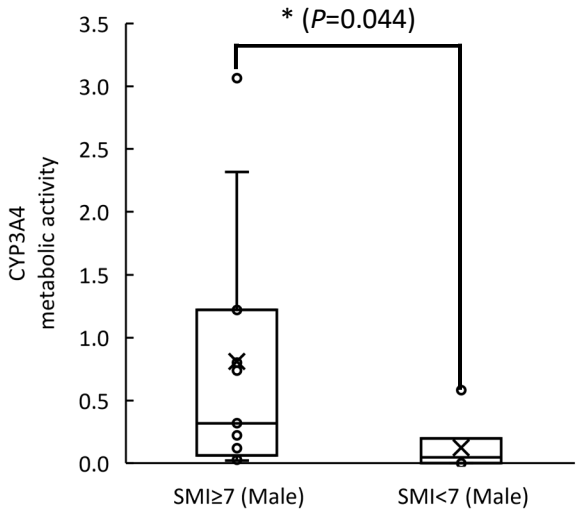

* (P=0.0017)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
CYP2C19
metabolic activity
HSI<36
HSI≥36

Figure 3


* (P=0.044)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
CYP3A4
metabolic activity
SMI≥7 (Male)
SMI<7 (Male)