Title:

Effects of feeding condition on the myocardial and hepatic accumulation of radioiodine-labeled BMIPP in mice

Short Title (27 / 30 characters):

BMIPP and feeding condition

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Abstract (294 / 350 words)

Objective:^{123}I-15-(*p*-iodophenyl)-3(R,S)-methylpentadecanoic acid ([^{123}I]BMIPP), a fatty acid analog, is widely used for the diagnosis of cardiac diseases. Feeding condition is one of the important factors in the myocardial fatty acid uptake, which may also affect myocardial accumulation of [^{123}I]BMIPP and image quality of [^{123}I]BMIPP scintigraphy. However, the relationship between the myocardial accumulation of [^{123}I]BMIPP and the feeding condition is not entirely clear. Therefore, we determined the myocardial accumulation of [^{125}I]BMIPP in mice at various metabolic statuses induced by fasting in comparison with the hepatic accumulation.

Methods: Fed or fasted (6, 12, and 24 h fasted) mice were intravenously injected with [^{125}I]BMIPP (35.2–75.0 kBq, 4 nmol). Radioactivities in the heart and liver were measured at 1, 5, 10, 30, 60, and 120 min after the injection (n = 5–15/time point for each group), and then the heart-to-liver (H/L) ratios were calculated.

Results: The myocardial accumulation level of [^{125}I]BMIPP in the fed group was almost the same as that in the 6 h fasted group at each time point, although it was decreased by 12 and 24 h fasting. The H/L ratios of [^{125}I]BMIPP accumulation level were significantly decreased by fasting (1.92 ± 0.22, 1.45 ± 0.13, 1.12 ± 0.13, and 0.91 ± 0.15 at 10 min, and 3.30 ± 0.62, 2.09 ± 0.35, 1.79 ± 0.34, and 1.27 ± 0.06 at 30 min after the injection

respectively for the fed group and the 6, 12, and 24 h fasted groups; $p < 0.0001$), largely owing to the increase in the hepatic accumulation level in the fasting groups.

Conclusion: Although short-period (6 h) fasting did not affect the myocardial accumulation level of [^{125}I]BMIPP, the hepatic accumulation level was increased. The present results indicate that the fed condition may provide higher-contrast images in myocardial [^{123}I]BMIPP scintigraphy.

Key Words (three to five):

[$^{123/125}$I]BMIPP / nuclear imaging / cardiology

Introduction

Fatty acids are a major energy source in the heart, and a decline in fatty acid utilization is caused by certain cardiac diseases. ^{123}I-15-(*p*-iodophenyl)-3(R,S)-methylpentadecanoic acid ([^{123}I]BMIPP), a branched-chain fatty acid analog, is thus widely used to assess cardiac fatty acid metabolism in the diagnosis of cardiac diseases. In particular, the usefulness for diagnosing ischemic heart disease, cardiomyopathy, heart failure, and myocardial viability has been recognized (1,2). However, it is of importance to establish an appropriate imaging protocol to obtain high quality images, as the diagnostic accuracy may be affected by the image quality. Actually, image contrast of [^{123}I]BMIPP scintigraphy is often deteriorated due to the low accumulation of [^{123}I]BMIPP in the heart and/or the high accumulation in the liver and blood.

The heart utilizes various circulating energy substrates (e.g., glucose, fatty acids, ketone bodies, and lactate) for energy production. Under fed condition, glucose is preferentially utilized, whereas fatty acids are alternatively utilized under fasted condition (3,4). Feeding condition is one of the important factors in the myocardial energy metabolism including fatty acid metabolism. Accordingly, feeding condition may also affect the myocardial accumulation of [^{123}I]BMIPP. Indeed, in many cases, [^{123}I]BMIPP is injected under fasting condition, anticipating higher myocardial accumulation levels of

[^{123}I]BMIPP. On the other hand, it has been reported that fasting before [^{123}I]BMIPP scintigraphy has little effect on the quality of myocardial images in healthy volunteers (5). Thus, the relationship between the myocardial accumulation of [^{123}I]BMIPP and the feeding condition is not entirely clear. Furthermore, we previously found that the hepatic accumulation level of [^{125}I]BMIPP was increased by fasting in mice (6), which indicated that fasting before [^{123}I]BMIPP scintigraphy may deteriorate the image contrast obtained.

Therefore, in this study, we determined the myocardial accumulation of [^{125}I]BMIPP in mice at various metabolic statuses induced by fasting in comparison with the hepatic accumulation to clarify the effects of feeding condition on the myocardial accumulation and image quality of [^{123}I]BMIPP scintigraphy.

Material and methods

Biodistribution of [^{125}I]BMIPP

All tissue samples were collected in our previous study (6), in which animal care and all experimental procedures were performed with the approval of the Animal Care Committee of Hokkaido University. Male C57BL/6J mice 15 weeks of age were purchased from CLEA JAPAN Inc. (Tokyo, Japan). The mice were housed and acclimatized under a 12 hour light-dark cycle (lights on from 8:00 a.m. to 8:00 p.m.) with *ad libitum* access to standard pelleted diet (MF, Oriental Yeast Co., Ltd., Tokyo, Japan) and water in a temperature controlled facility. A simple schematic of the experimental protocols is shown in Fig. 1. All biodistribution experiments were started at 10:00 a.m., that is, the time period of fasting is from 4:00 a.m. to 10:00 a.m., from 10:00 p.m. to 10:00 a.m. and from 10:00 a.m. on the previous day to 10:00 a.m. respectively for the 6, 12, and 24 h fasted group. Fed (fed *ad libitum*) or fasted (6, 12, and 24 h fasted) mice were intravenously injected with 100 μl of [^{125}I]BMIPP (35.2–75.0 kBq, 4 nmol). One, 5, 10, 30, 60, and 120 min after the injection, the mice were sacrificed by whole blood collection during isoflurane anesthesia and the heart and liver were rapidly removed and weighed (n = 5–15/time point for each group). The radioactivities in the heart, liver, and blood were counted using a well-type scintillation counter (1480 WIZARD 3″; Perkin Elmer,

Waltham, MA). The accumulation level of [^{125}I]BMIPP was calculated as the percentage of the injected dose per gram (%ID/g) of tissue and normalized with the animal's body weight (%ID/g/kg). The remaining blood samples were used for biochemical examination [insulin, β-hydroxybutyrate (a most prevalent in serum and accounts for about 75% of all ketone bodies), non-esterified fatty acids (NEFAs), triglycerides (TGs), total cholesterol, free cholesterol, and high-density lipoprotein (HDL) cholesterol].

Measurement of blood parameters

Before the [^{125}I]BMIPP injection, the blood glucose levels of fed (fed *ad libitum*) or fasted (6, 12, and 24 h fasted) mice were measured by a glucometer (Accu-Chek; Roche Diagnostics, Tokyo, Japan) using the blood drawn from the tail vein without anesthesia.

Insulin, β-hydroxybutyrate, NEFAs, TGs, total cholesterol, free cholesterol, and HDL cholesterol levels were measured using LBIS Mouse Insulin ELISA kit (Shibayagi, Gunma, Japan), β-hydroxybutyrate Assay Kit (BioVision, Milpitas, CA, USA) and NEFA C-test Wako, Triglyceride E-test Wako, Cholesterol E-test Wako, Free Cholesterol E-test Wako and HDL-Cholesterol E-test Wako (FUJIFILM Wako Pure Chemical, Osaka, Japan), respectively.

Data Analysis

Numeric parameters were expressed as mean ± SD. One-way ANOVA followed by a Bonferroni post-hoc test was carried out to evaluate the effects of feeding status on body weight and each blood parameter (Table 1). Two-way factorial ANOVA was performed to evaluate the radioactivity in the heart, the heart-to-liver (H/L) ratio, and heart-to-blood (H/B) ratio in relation to their time course and feeding status. One-way ANOVA followed by a Bonferroni post-hoc test was carried out to assess the significance of difference among the groups at each time point (Figs. 2 and 3, Supplemental Tables 1 and 2). A $p<0.0083$ (i.e., 0.05/6) was considered statistically significant.

Results

Animal body weight and blood parameters

The body weight and blood parameters of each group are shown in Table 1. Depending on the fasting time, the body weight and blood glucose level were decreased, whereas the β-hydroxybutyrate level was increased. The NEFA level was increased by 6 and 12 h fasting. As for the body weight and insulin level, there were no significant differences between the fed and 6 h fasted groups. The TG and cholesterol levels in plasma are also shown in Table 1.

Myocardial accumulation of [^{125}I]BMIPP in each group

Figure 2 and Supplemental Table 1 show the myocardial accumulation of [^{125}I]BMIPP at each time point. In the fed group, the myocardial accumulation level of [^{125}I]BMIPP peaked at 5 min and then gradually decreased. The myocardial accumulation of [^{125}I]BMIPP in the 6 h fasted group showed similar time courses to the fed group, and there were no significant differences at each time point. On the other hand, the myocardial accumulation levels of [^{125}I]BMIPP in the 12 and 24 h fasted groups were significantly lower than those in the fed group at most time points.

H/L and H/B ratios of [^{125}I]BMIPP accumulation level in each group

Figures 3a and 3b, and Supplemental Table 2 show the H/L and H/B ratios of [^{125}I]BMIPP accumulation level at each time point. The H/L ratios of the fed group gradually increased and reached a plateau at 30 min. The H/L ratios were significantly decreased by 6 h fasting at 10, 30, and 120 min after the injection. Additionally, the H/L ratios of the 12 and 24 h fasted group were significantly lower than that of the fed group at each time point. The H/B ratio of the fed group peaked at 5 min and then rapidly decreased. The H/B ratios were significantly increased by 6 h fasting at 5 and 10 min after the injection. However, there were no significance differences among the four groups 30 min after the injection.

Discussion

Although the myocardial accumulation of [$^{123/125}$I]BMIPP has been evaluated in a considerable number of studies, the effects of fasting have been evaluated in only a few studies. In this study, we determined the myocardial accumulation of [^{125}I]BMIPP in mice at various metabolic statuses induced by fasting in comparison with the hepatic accumulation and blood radioactivity. The results can be summarized as follows. A short-period (6 h) fasting did not affect the myocardial accumulation level of [^{125}I]BMIPP, whereas a longer period (12 and 24 h) of fasting decreased the myocardial accumulation level. On the other hand, the hepatic accumulation level of [^{125}I]BMIPP was increased depending on the fasting time (Supplemental Fig. 1a) (6). Consequently, the H/L ratio was decreased by fasting. The present results indicate that the fed condition may provide higher-contrast images in myocardial [^{123}I]BMIPP scintigraphy.

It is well known that a short period of fasting increases myocardial fatty acid utilization (7,8). Thus, the myocardial accumulation level of [^{125}I]BMIPP is expected to be increased by 6 h fasting. However, 6 h fasting did not affect the myocardial accumulation of [^{125}I]BMIPP (Fig. 2). It is also known that the myocardial accumulation of [^{125}I]BMIPP is affected by endogenous fatty acid levels (9,10). The levels of endogenous fatty acids are low in the postprandial state, whereas they are increased by fasting. Indeed, 6 h fasting

significantly increased the blood NEFA level in our mice (Table 1). Taken together, increased fatty acid utilization and competition with endogenous fatty acids may explain our results indicating that the myocardial accumulation of [^{125}I]BMIPP was not changed by 6 h fasting. Our results are consistent with these previous studies in human (11). Also in humans, it has been reported that plasma NEFA level increases by fasting (11). Kurata and colleagues reported that the early myocardial [^{123}I]BMIPP uptake negatively correlated with serum FFA levels (9). In addition, fasting before [^{123}I]BMIPP scintigraphy has little effect on the quality of myocardial images in healthy volunteers (5). Our results may partly explain these phenomena, and also provide important information for basic and clinical research using [$^{123/125}$I]BMIPP. On the other hand, the myocardial accumulation level of [^{125}I]BMIPP was decreased by long-period (12 and 24 h) fasting, which decreased body weight. These results may be caused by the increased competition between [^{125}I]BMIPP and markedly elevated endogenous energy substrates including ketone bodies (7). Thus, our present results indicate that fasting before [^{123}I]BMIPP scintigraphy may not be important for the myocardial accumulation of [^{123}I]BMIPP.

In clinical settings of [^{123}I]BMIPP scintigraphy, hepatic radioactivity often affects the myocardial image quality. In our mice injected with [^{125}I]BMIPP, the hepatic radioactivity was higher in the fasted mice than in the fed mice, and this tendency was dependent on

the fasting time (Supplemental Fig. 1a) (6). A major factor involved in the increased hepatic radioactivity may be the suppressed secretion of radioactive TGs from the liver (6). Consequently, the H/L ratios of [^{125}I]BMIPP accumulation level were decreased by fasting (Fig. 3a), and this was particularly notable at time points later than 10 min after the injection. In terms of H/L, a fed condition is appropriate for obtaining higher-contrast images in myocardial [^{123}I]BMIPP scintigraphy, which also eases the patient's burden.

Unlike the H/L ratio, the H/B ratios were significantly increased by 6 h fasting at 5 and 10 min after the injection (Fig. 3b, Supplemental Fig. 1b). In many cases, [^{123}I]BMIPP scintigraphy is obtained 15 to 30 min (early) and/or 3 h (delay) after tracer administration in clinical practice (1,12–14). This information taken together with our results indicate that short-period fasting may be appropriate for early (15 to 30 min) [^{123}I]BMIPP scintigraphy. However, the fed group also achieved substantially high H/B ratios at these time points. Accordingly, the fed condition may be also feasible for [^{123}I]BMIPP myocardial scintigraphy, considering the easing of the patient's burden.

Note that 6 h fasting may still be an immoderate fasting state for mice. However, the blood glucose, insulin, NEFA, and β-hydroxybutyrate levels were within the normal ranges, and body weight did not change in our 6 h fasting mice (Table 1). Typical [^{123}I]BMIPP scintigraphy in many clinical studies are performed under approximately 12

h fasted condition, that is, no breakfast and/or overnight fasting (5, 9, 12). As described above, our results are consistent with a previous study conducted under fasted at least 12 h or longer condition in healthy volunteers (5). Thus, the 6 h fasting condition in our mice seems to be a mimic of a moderate fasting state in clinical settings, although further investigations on shorter fasting times (< 6 h) and clinical studies should provide additional information about the effects of feeding condition on the myocardial accumulation of [^{125}I]BMIPP. Furthermore, it is also important to scientifically relate the fasting conditions, blood parameters, and myocardial and hepatic [^{123}I]BMIPP accumulation in animals models and human.

[^{123}I]BMIPP scintigraphy is particularly effective for diagnosing various cardiac diseases. It is well established that aging is one of the important risk factors for these diseases (15). Therefore, a person who needs the [^{123}I]BMIPP scintigraphy are often after post-middle age. Fifteen-week-old mice used in our study corresponds to post-middle age in human. Thus, our results may also be useful for the above further investigation.

CONCLUSION

Short-period (6 h) fasting did not affect the level of myocardial accumulation of [^{125}I]BMIPP, whereas that of hepatic accumulation was increased. Consequently, the H/L ratio was decreased by fasting, indicating that the fed condition may provide higher-contrast images in myocardial [^{123}I]BMIPP scintigraphy. Thus, the present results provide important information not only for basic research, but also for obtaining [^{123}I]BMIPP scintigraphy images of adequate quality in clinical settings.

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TABLE 1 Animal body weight and blood parameters in each group

	Fed (n=90)	**6 h Fasted (n=30)**	**12 h Fasted (n=35)**	**24 h Fasted (n=40)**
Body weight (g)	27.3±1.4	26.5±1.9	25.1±1.4*†	23.8±1.5*†‡
Blood glucose level (mg/dL)	197.6±34.4	156.6±20.9*	107.2±26.5*†	95.2±14.9*†
Insulin level (ng/mL)	0.86±0.57	0.63±0.26	0.32±0.11*†	0.37±0.05*
β-Hydroxybutyrate level (mM)	0.48±0.23	0.76±0.31*	1.13±0.30*†	3.15±1.00*†‡
NEFA level (mM)	1.21±0.31	1.37±0.16*	1.48±0.35*	1.21±0.19‡
TG level (mM)	0.81±0.27	0.66±0.19*	0.75±0.14	1.00±0.28*†‡
Total CHO level (mM)	1.44±0.27	1.28±0.20*	1.43±0.18	1.58±0.28*†
Free CHO level (mM)	0.21±0.11	0.08±0.06*	0.15±0.06*†	0.25±0.05†‡
HDL level (mM)	0.96±0.25	0.84±0.26	1.21±0.30*†	1.14±0.61†

Data represent mean ± SD. One-way ANOVA showed significant changes in each parameter (body weight: $F = 55.30$, $p < 0.0001$; blood glucose level: $F = 163.27$, $p < 0.0001$; insulin level: $F = 18.10$, $p < 0.0001$; β-hydroxybutyrate level: $F = 248.30$, $p < 0.0001$; NEFA level: $F = 8.30$, $p < 0.0001$; TG level: $F = 10.42$, $p < 0.0001$; total CHO level: $F = 7.44$, $p < 0.0001$; free CHO level: $F = 22.37$, $p < 0.0001$; HDL level: $F = 7.84$, $p < 0.0001$) among the four groups. Therefore, significant changes were evaluated at each parameter. A $p < 0.0083$

(i.e., 0.05/6) was considered statistically significant; *, vs. fed group; †, vs. 6 h fasted group; ‡, vs. 12 h fasted group.

FIGURE LEGENDS

FIGURE 1. Experimental protocols of biodistribution study

FIGURE 2. Myocardial accumulation of [^{125}I]BMIPP in each group

Data represent mean ± SD (%ID/g/kg, n=5–15/time point for each group). Two-way factorial ANOVA showed significant changes in myocardial accumulation of [^{125}I]BMIPP with the feeding condition (F = 42.36; p < 0.0001) and each time point (F = 39.77; p < 0.0001). Interaction between the effects of the feeding condition and the time point was observed (F = 3.67; p < 0.0001). When an interaction was confirmed, significant changes were evaluated at each time point. *, vs. fed group; †, vs. 6 h fasted group; ‡, vs. 12 h fasted group.

FIGURE 3. Heart-to-liver (H/L) (a) and heart-to-blood (H/B) (b) ratios of [^{125}I]BMIPP accumulation level in each group

Data represent mean ± SD (n = 5–15/time point for each group). Two-way factorial ANOVA showed significant changes in H/L and H/B ratios with the feeding condition (F = 123.20; $p < 0.0001$ and F = 8.81; $p < 0.0001$) and each time point (F = 54.15; $p < 0.0001$ and F = 146.48; $p < 0.0001$). Interaction between the effects of the feeding condition and the time point was observed (F =8.93; $p < 0.0001$ and F =6.71; $p < 0.0001$). When an interaction was confirmed, significant changes were evaluated at each time point. *, vs. fed group; †, vs. 6 h fasted group; ‡, vs. 12 h fasted group.

Fig. 1

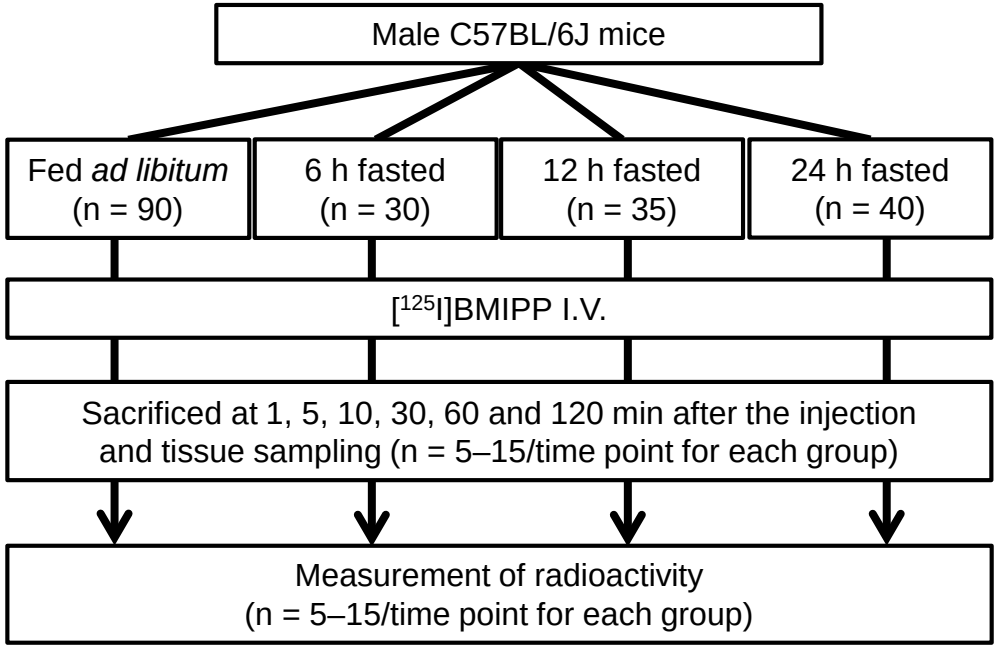

Male C57BL/6J mice
Fed *ad libitum* (n = 90)
6 h fasted (n = 30)
12 h fasted (n = 35)
24 h fasted (n = 40)
[^{125}I]BMIPP I.V.
Sacrificed at 1, 5, 10, 30, 60 and 120 min after the injection and tissue sampling (n = 5–15/time point for each group)
Measurement of radioactivity (n = 5–15/time point for each group)

Fig. 2

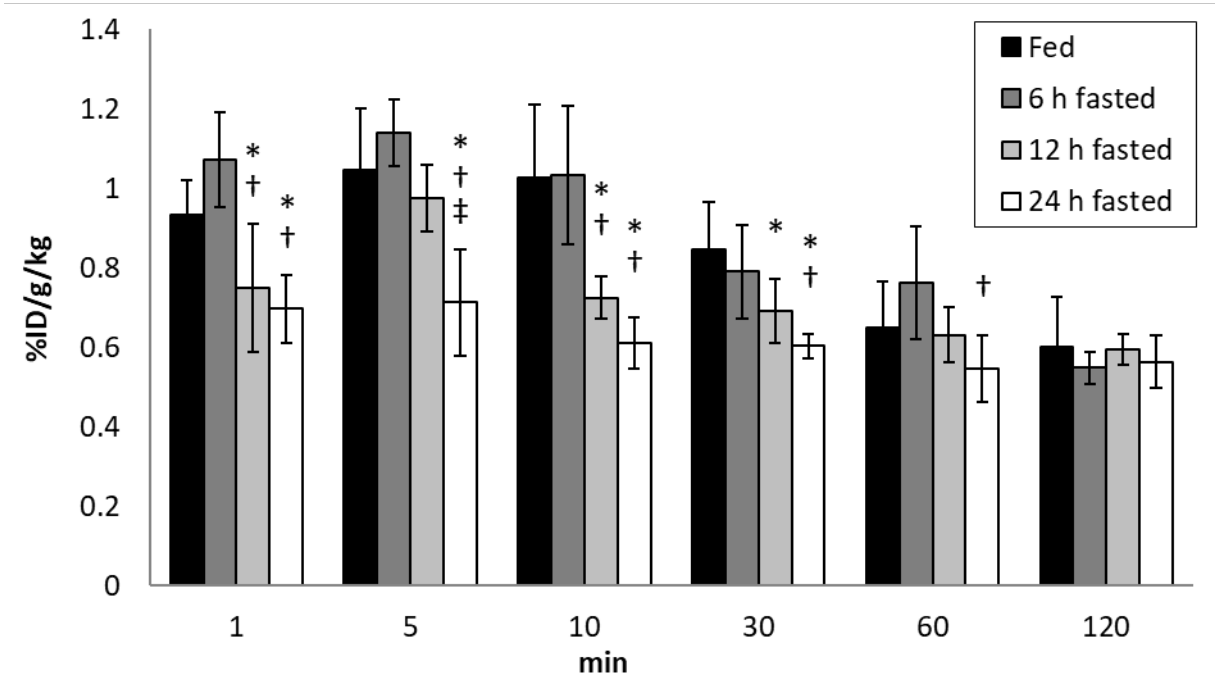
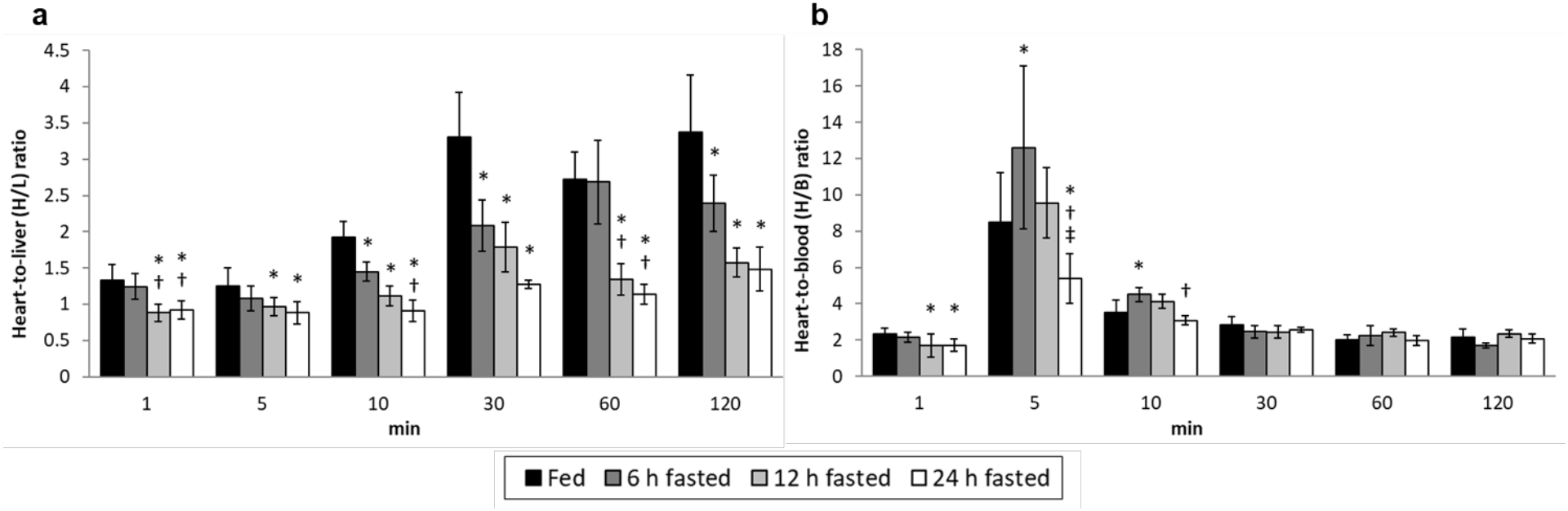

1.4
1.2
1
0.8
0.6
0.4
0.2
0
%ID/g/kg
1
5
10
30
60
120
min
Fed
6 h fasted
12 h fasted
24 h fasted

Fig. 3


a
b
Heart-to-liver (H/L) ratio
Heart-to-blood (H/B) ratio
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
18
16
14
12
10
8
6
4
2
0
1
5
10
30
60
120
min
Fed
6 h fasted
12 h fasted
24 h fasted