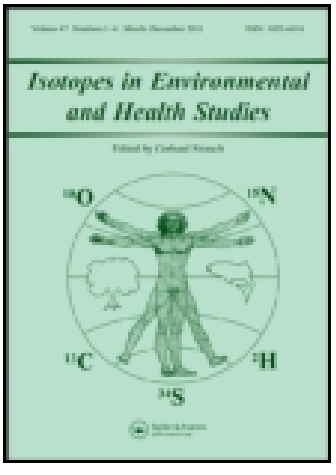

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Effect of alcohol consumption on the liver detoxication capacity as measured by [$^{13}C_2$]aminopyrine and L-[1-^{13}C]phenylalanine breath tests

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Effect of alcohol consumption on the liver detoxication capacity as measured by [$^{13}C_2$]aminopyrine and L-[1-^{13}C]phenylalanine breath tests

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The aim of this study was to investigate the hepatic microsomal and cytosolic functions by using the $^{13}CO_2$ breath test in healthy subjects either before or after consumption of red wine. Twelve adults received [$^{13}C_2$]aminopyrine and L-[1-^{13}C]phenylalanine together with a standardised dinner. Expired air samples were taken over 6 h. After a wash-out period, the subjects consumed 0.4 ml ethanol per kg per day together with dinner over a 7.5-day period on average. Thereafter, ^{13}C-tracer administration was repeated under identical conditions. The $^{13}CO_2$ enrichments were measured by isotope ratio mass spectrometry. The mean cumulative percentage ^{13}C-dose recovery after administration of [$^{13}C_2$]aminopyrine and L-[1-^{13}C]phenylalanine either without or with red wine consumption amounted to 17.0 ± 4.4 vs. $14.7 \pm 3.1\%$ ($p = 0.170$) and 14.0 ± 2.8 vs. $11.5 \pm 3.9\%$ ($p = 0.084$), respectively. Moderate alcohol consumption does not induce significant short-term changes of the microsomal and the cytosolic function of the human liver in healthy subjects.

Keywords: alcohol; ^{13}C; $^{13}CO_2$ breath tests; diagnostic isotope application in medicine; liver function; human; [$^{13}C_2$]aminopyrine; L-[1-^{13}C]phenylalanine

1. Introduction

Various ^{13}C-labelled substrates have been used to characterise different liver dysfunctions and to explore the harmful effects of excessive alcohol drinking on specific hepatic functions [1,2]. After the hepatic degradation of the orally administered ^{13}C-labelled tracer substances, the resulting $^{13}CO_2$ exhaled in breath generally reflects the detoxication capacity of the liver. $^{13}CO_2$ breath tests are classified according to the hepatic enzyme activities involved in the metabolism [3]. To date, $^{13}CO_2$ breath tests published on liver metabolism mainly focused on the investigation of specific hepatic functions in patients suffering from acute liver cirrhosis and steatosis caused by alcohol abuse [1,2]. However, little is known about short-term functional microsomal, cytosolic,

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and mitochondrial changes in response to socially consumed amounts of alcohol, which is of great interest for the public health system, physicians, scientists, and for the consumers as well.

In our previous study, we investigated the hepatic microsomal and mitochondrial functions by using the [^{13}C]methacetin- and L-[methyl-^{13}C]methionine-$^{13}CO_2$ breath test in healthy subjects either before or after consumption of red wine in moderate dosages. After administration of both ^{13}C-labelled substrates either without or with red wine consumption, the resulting cumulative percentage ^{13}C-dose recovery (CPDR) revealed no statistically significant differences [3]. In continuation of these investigations, the aim of the present follow-up study was to explore further hepatic microsomal and cytosolic detoxication parameters by using the [$^{13}C_2$]aminopyrine- and the L-[1-^{13}C]phenylalanine-$^{13}CO_2$ breath test, respectively, either before or after continuous, moderate ethanol-induced oxidative stress by red wine consumption in human beings. Furthermore, the objective of this study was to verify our recently published results.

2. Material and methods

2.1. *Subjects*

Twelve healthy adults (six males, six females, aged 21–32, mean body weight: 66.5 kg, mean body mass index: 22.0 kg/m^2) volunteered for this study. None of the subjects were receiving any medication or had had a history of gastrointestinal or of hepatic diseases. They were in good health throughout the study. All volunteers rarely consumed alcoholic beverages (once only per week) prior to study begin. Moreover, they were instructed to not consume alcoholic beverages for 10 days prior to study begin. Furthermore, none of the subjects used nicotine and none of the female volunteers were taking oral contraceptives.

The testing protocol was approved by the Committee on Ethics of the Faculty of Medicine of the University of Rostock (registration number: amendment of II HV 10/2002). Written consent was obtained from all subjects.

2.2. *Study protocols*

In a blind, randomised cross-over study one-half of the subjects (three males and three females) received [$^{13}C_2$]aminopyrine and 5 days later L-[1-^{13}C]phenylalanine (99 at%^{13}C each, (Campro Scientific, Berlin, Germany) and vice versa. Both ^{13}C-tracer substances were given together with dinner at 5 p.m. as a single oral pulse in a dosage of 2 mg/kg body weight corresponding to a ^{13}C-excess dose of 16.77 and 11.77 μmol/kg, respectively. The dinner was made up of two rye bread slices (169 kcal) with butter (111 kcal), cheese (125 kcal), ham (101 kcal), and one glass of apple juice (92 kcal). The ^{13}C-tracer substances were strewn on a small area of the butter to ingest the label with one bite. No other food was allowed after dinner. Two expired breath samples obtained by direct exhalation into Exetainers^{TM} (IVA Analysentechnik, Meerbusch, Germany) were taken in 15 and 30 min intervals over a period of 6 h for measuring $^{13}CO_2$ enrichment [3]. Thereafter, the subjects consumed 0.4 ml ethanol per kg per day, equivalent to 3.5 ml red wine per kg per day (dry Bordeaux, 11.5 vol% ethanol) within 30 min together with dinner over a timespan of 10 days. After 5 and 10 days, the cross-over procedure of the alternate [$^{13}C_2$]aminopyrine and L-[1-^{13}C]phenylalanine administration, respectively, was repeated under identical conditions. The composition of the dinner, the ^{13}C-tracer administration, the time-interval of taking $^{13}CO_2$ breath samples, the duration of red wine consumption, and the red wine dosage consumed by the volunteers were identical with our recently published study [3]. In order to maintain the non-invasive character of our study, blood alcohol concentrations were not measured.

2.3. *Analytical techniques*

The $^{13}CO_2$ enrichments in breath were measured by isotope ratio mass spectrometry (Tracer mass 20-20^{TM}, SerCon, Crewe, UK) as detailed previously [4,5]. The data were expressed either as enrichment $\delta^{13}C$ over baseline (DOB) or as CPDR in breath [6,7].

2.4. *Cumulative percentage ^{13}C-dose recovery*

The ^{13}C-excess dose and the CPDR were calculated according to the equations described earlier [5,8,9].

2.5. *Statistical method*

The Wilcoxon test was used for statistic analysis on program SPSS 14.0 for Windows. All results are quoted as means ± SD.

3. Results

Figures 1 and 2 show the $^{13}CO_2$-exhalation rates and the resulting CPDR, respectively, over 6 h after oral [$^{13}C_2$]aminopyrine and L-[1-^{13}C]phenylalanine administration, respectively, prior and after the averaged 7.5-day period of red wine consumption. After [$^{13}C_2$]aminopyrine administration, the mean maximum $^{13}CO_2$-exhalation rates either without or after red wine consumption of 4.1 and 3.4 %/h, respectively, were reached after 3.0 and 3.5 h, respectively, and were still detectable after 6 h (Figure 1). The corresponding calculated CPDR amounted to 17.0 ± 4.4 and 14.7 ± 3.1% and were not significantly different $p = 0.170$. After L-[1-^{13}C]phenylalanine administration, the mean maximum $^{13}CO_2$ exhalation rates either without or after red wine consumption of 3.6 and 3.2 %/h, respectively, were reached after 3.0 h and were still detectable after 6 h (Figure 2). The corresponding calculated CPDR amounted to 14.0 ± 2.8 and 11.5 ± 3.9 % and were not significantly different $p = 0.084$. There was no significant gender difference in the

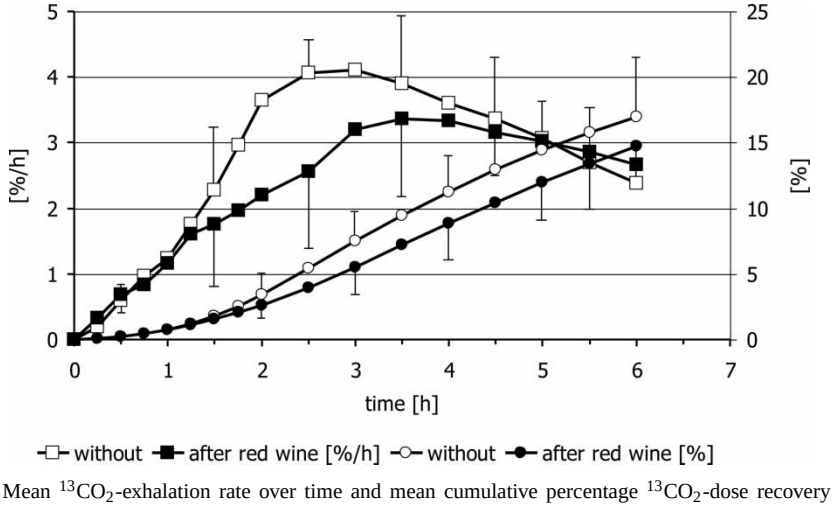

Figure 1. Mean $^{13}CO_2$-exhalation rate over time and mean cumulative percentage $^{13}CO_2$-dose recovery after oral [$^{13}C_2$]aminopyrine administration either without or after a 7.5-day period on average of red wine consumption in 12 healthy subjects.

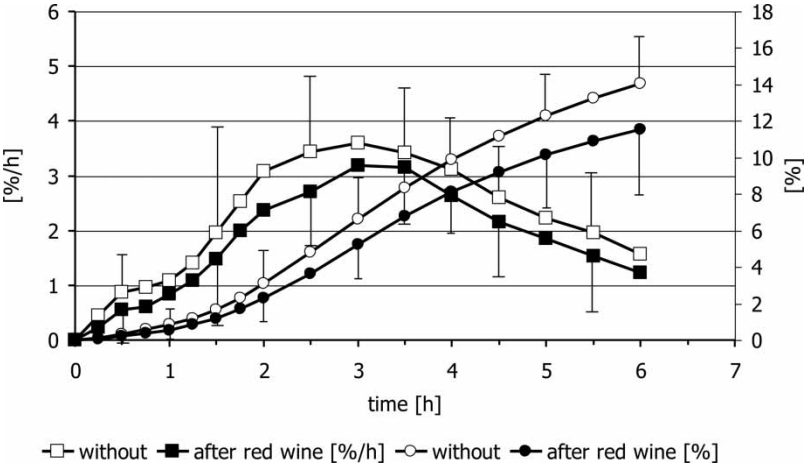

Figure 2. Mean $^{13}CO_2$-exhalation rate over time and mean cumulative percentage $^{13}CO_2$-dose recovery after oral [1-^{13}C]phenylalanine administration either without or after a 7.5-day period on average of red wine consumption in 12 healthy subjects.

Table 1. Mean cumulative percentage $^{13}CO_2$-dose recovery (CPDR) after oral [$^{13}C_2$]aminopyrine- and L-[1-^{13}C]phenylalanine-administration without and after a 7.5-day period on average of red wine consumption in six male and six female healthy subjects

		CPDR (%)			
		[$^{13}C_2$]Aminopyrine		L-[1-^{13}C]Phenylalanine	
Subjects	Gender	Without red wine	After red wine	Without red wine	After red wine
AB	M	21.3	16.5[a]	15.3	11.0[b]
KR	M	14.7	16.1[a]	14.3	22.2[b]
SK	M	18.6	18.9[b]	16.8	11.7[a]
CS	M	8.5	13.3[b]	15.7	8.3[a]
ES	M	18.7	13.1[b]	9.2	7.2[a]
HW	M	21.7	9.4[b]	8.9	10.6[a]
Mean	M	17.3[c]	14.6[c]	13.4[d]	11.8[d]
SD		4.5	3.0	3.1	4.9
MB	F	18.4	13.7[a]	15.4	9.8[b]
DD	F	14.2	10.5[a]	12.9	8.7[b]
JK	F	18.1	15.6[a]	13.5	13.8[b]
VS	F	18.3	16.3[a]	13.2	14.6[b]
BB	F	22.6	12.6	13.9	11.7[a]
NH	F	8.8	20.4	19.2	8.5[a]
Mean	F	16.7[e]	14.9[e]	14.7[f]	11.2[f]
SD		4.3	3.1	2.2	2.4
General mean	Both sexes	17.0[g]	14.7[g]	14.0[h]	11.5[h]
SD		4.4	3.1	2.8	3.9

[a]Five days. [$^{13}C_2$]aminopyrine: 5 vs. 10 days, $p = 0.753$.
[b]Ten days. L-[1-^{13}C]phenylalanine: 5 vs. 10 days, $p = 0.116$.
[c][$^{13}C_2$]aminopyrine (M): without red wine vs. after red wine, $p = 0.400$.
[d]L-[1-^{13}C]phenylalanine (M): without red wine vs. after red wine, $p = 0.463$.
[e][$^{13}C_2$]aminopyrine (F): without red wine vs. after red wine, $p = 0.345$.
[f]L-[1-^{13}C]phenylalanine (F): without red wine vs. after red wine, $p = 0.116$.
[g][$^{13}C_2$]aminopyrine (both sexes): without red wine vs. after red wine, $p = 0.170$.
[h]L-[1-^{13}C]phenylalanine (both sexes) without red wine vs. after red wine, $p = 0.084$.

CPDR values after [$^{13}C_2$]aminopyrine and L-[1-^{13}C]phenylalanine administration either without or with red wine drinking, respectively (Table 1). Furthermore, the different duration of red wine consumption over 5 and 10 days showed no significant differences of the CPDR values, neither after [$^{13}C_2$]aminopyrine nor L-[1-^{13}C]phenylalanine administration, respectively (Table 1). The red wine consumption led to decreased but not statistically different CPDR values.

4. Discussion

Non-invasive breath tests with ^{13}C-labelled compounds have been proposed as non-invasive, sensitive, and accurate, being useful for the evaluation of specific liver functions [3]. [$^{13}C_2$]aminopyrine [10–14] and L-[1-^{13}C]phenylalanine [15–20] were found to be appropriate, reliable, and representative liver test substrates for evaluation of the microsomal and the cytosolic function, respectively, when used in human subjects. In this study, we used the combination of measuring the $^{13}CO_2$ exhalation after [$^{13}C_2$]aminopyrine and L-[1-^{13}C]phenylalanine administration either without or after ethanol-induced stress to healthy subjects to gain further insight into the hepatic changes that accompany the continuous consumption of red wine in moderate dosages. The [$^{13}C_2$]aminopyrine breath test has been mainly used in patients with acute chronic liver diseases (alcoholic and viral cirrhosis) and in patients undergoing major liver surgery (transplantation and partial hepatectomy) for assessing the hepatic microsomal cytochrome P450 metabolism [11,13]. After oral ingestion [$^{13}C_2$]aminopyrine is rapidly absorbed from the small bowel and is then metabolised by the liver. It subsequently undergoes enzyme-mediated N-demethylation and oxidation to [^{13}C]formaldehyde and aminoantipyrine. The resulting [^{13}C]formic acid is then converted to [^{13}C]bicarbonate and is finally exhaled as $^{13}CO_2$. We hypothesised that ethanol ingestion would decrease the ^{13}C-substrate oxidation since it inhibits the microsomal NADPH cytochrome reductase [2,3]. In several earlier published papers, [$^{13}C_2$]aminopyrine was given after an overnight fast [11,12]. In our study, the ^{13}C-tracer was administered together with dinner and the ^{13}C-exhalation was measured over 6 h to compensate the delayed degradation of the ^{13}C-labelled substrates caused by the simultaneous hepatic metabolism of the food components of the dinner [3]. Our calculated CPDR values were found to be similar to the data described by Van Vlierberghe *et al.* [12] for healthy controls. They investigated the microsomal degradation of 2 mg/kg [$^{13}C_2$]aminopyrine and described a significant drop in the CPDR values after acute consumption of 30 g ethanol in healthy controls (control vs. alcohol: male 20.2% vs. 20.0%, female 20.0% vs. 14.1%). However, it is difficult to compare the CPDR of our study with those of Van Vlierberghe *et al*. since they had applied a single ethanol drink after an overnight fast.

L-[1-^{13}C]phenylalanine represents an essential aromatic amino acid and has been mainly used in patients with end-stage liver disease [15]. It is degraded in the cytosol of hepatocytes catalysed by phenylalanine hydroxylase to [^{13}C]tyrosine, the rate limiting step of phenylalanine degradation. After transamination, the resulting 4-hydroxyphenylpyruvate is dioxygenated to homogentisic acid and $^{13}CO_2$, which is finally exhaled [16]. Our findings after a 6 h ^{13}C -exhalation period are difficult to compare with the data of other studies since different groups of subjects, $^{13}CO_2$-exhalation periods, ^{13}C-dosages and units, respectively, have been used. Lara Baruque *et al.* [18] found CPDR data of 7.5% 1 h after 100 mg L-[1-^{13}C]phenylalanine administration. Festi *et al.* [17] observed a CPDR of 5.4%/h in healthy adults, whereas Burke *et al*. [15] estimated 7.1%/h. However, comparable CPDR data of L-[1-^{13}C]phenylalanine administration after ethanol consumption are not available in healthy subjects. Figures 2 and 4 show that the CPDR did not reach a plateau level 6 h after [$^{13}C_2$]aminopyrine or [1-^{13}C]phenylalanine ingestion, indicating that the ^{13}C -label is temporarily trapped in the bicarbonate pool or in other metabolic pathways of the human body leading to an underestimation of the CPDR. Furthermore, we did not

observe significantly different CPDR values deriving either from [$^{13}C_2$]aminopyrine or from [1-^{13}C]phenylalanine between males and females (Table 1).

In our study, an average one-week period (7.5 days) of alcohol drinking was used to approximately adjust the volunteers to ethanol-induced metabolic steady-state conditions. As already discussed in our previous study, food, red wine, and both ^{13}C -tracer substances were deliberately given together to simulate a conventional dinner and to explore the impact of an alcoholic beverage on hepatic ^{13}C -tracer degradation, respectively. The simultaneous ^{13}C -tracer ingestion together with food intake could have led to a delayed ^{13}C -tracer resorption [3,21]. This study protocol has been chosen to keep the design as close as possible to our previous study. We primarily intend to study chronic effects of short-term moderate alcohol consumption on liver function. However, the red wine intake on the day of ^{13}C -tracer administration represents partly acute effects of alcohol on the microsomal and cytosolic handling of substrates administered in the food matrix.

When considering the results with those of our previous study, a tendency among the dry period and the red wine period becomes obvious. The administration of four different substrates, L-[methyl-^{13}C]methionine, [^{13}C]methacetin, [$^{13}C_2$]aminopyrine, and L-[1-^{13}C]phenylalanine representative for the hepatic mitochondrial, microsomal and cytosolic metabolism, respectively, resulted in decreased but not statistically significant cumulative percentage dose recovery after red wine consumpion to healthy adults. To what extent this typical response can be explained by an inhibited enzyme activity caused by the impact of alcohol or by addiction effects remains open. In four similar studies (two unpublished till now) we investigated a total of 72 volunteers with moderate alcohol consumption by using eight different ^{13}C -labelled substrates including [1-^{13}C]galactose, [trimethyl-$^{13}C_3$]caffeine, [1-^{13}C]-α-keto-isocaproic acid, and [1-^{13}C]ethanol. We never observed significant differences [22]. However, we are aware that the relatively low number of subjects involved in the present study as well as the variation of the results, possibly caused by altered physical activities of the volunteers and unpredictable variations of gastric emptying, affects our argumentation. When summarising, the data of the present study confirm our previous findings that a moderate social alcohol consumption over a relatively short period does not induce significant short-term changes of both the microsomal and the cytosolic function of the human liver in healthy subjects. Further red wine studies with other ^{13}C -labelled liver test substrates are in preparation.

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