# An investigation into potential gender-specific differences in myocardial triglyceride content assessed by [1]H-Magnetic Resonance Spectroscopy at 3Tesla

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## Abstract

**Objective:** Over the past decade, myocardial triglyceride content has become an accepted biomarker for chronic metabolic and cardiac disease. The purpose of this study was to use proton (hydrogen 1)-magnetic resonance spectroscopy (^{1}H-MRS) at 3Tesla (3 T) field strength to assess potential gender-related differences in myocardial triglyceride content in healthy individuals.

**Methods:** Cardiac MR imaging was performed to enable accurate voxel placement and obtain functional and morphological information. Double triggered (i.e., ECG and respiratory motion gating) ^{1}H-MRS was used to quantify myocardial triglyceride levels for each gender. Two-sample t-test and Mann-Whitney U-test were used for statistical analyses.

**Results:** In total, 40 healthy volunteers (22 male, 18 female; aged >18 years and age matched) were included in the study. Median myocardial triglyceride content was 0.28% (interquartile range [IQR] 0.17–0.42%) in male and 0.24% (IQR 0.14–0.45%) in female participants, and no statistically significant difference was observed between the genders. Furthermore, no gender-specific difference in ejection fraction was observed, although on average, male participants presented with a higher mean ± SD left ventricular mass (136.3 ± 25.2 g) than female participants (103.9 ± 16.1 g).

**Conclusions:** The study showed that ^{1}H-MRS is a capable, noninvasive tool for acquisition of myocardial triglyceride metabolites. Myocardial triglyceride concentration was shown to be unrelated to gender in this group of healthy volunteers.

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## Introduction

A number of studies have demonstrated the potential of proton (hydrogen 1)-magnetic resonance spectroscopy (^{1}H-MRS) for the noninvasive assessment of myocardial triglyceride content.[1,2] The availability of high field (3 T) magnetic resonance (MR) systems in clinical practice has facilitated precise measurements of myocardial tissue metabolites, as signal intensity is augmented with field strength.[3,4]

The use of double triggered (i.e., electrocardiogram [ECG] and respiratory motion gating) ^{1}H-MRS increases the potential clinical application of the technique, particularly for cardiac examinations.[5] Indeed, a strong correlation between *in vivo* ^{1}H-MRS and *ex vivo* myocardial triglyceride measurements has been demonstrated.[6]

Over the past decade, *in vivo* evaluation of myocardial triglyceride content has become an accepted biomarker for chronic metabolic and cardiac disease.[5,7,8] Body mass index (BMI), hepatic triglyceride content, visceral fat and metabolic disease have been shown to be related to myocardial triglyceride concentration.[8] However, little information is available about possible differences in myocardial triglyceride concentrations between men and women.[9]

The aim of this present study was to analyse the concentration of myocardial triglyceride metabolites in healthy volunteers, to assess any potential gender difference.

## Subjects and methods

### Study population

Male and female healthy volunteers $\geq 18$ years of age were included in this study. Exclusion criteria included history of cardiac disease, metabolic disease (in particular diabetes mellitus), presence of implanted devices possibly limiting cardiac imaging and spectroscopy, pregnancy and fear of confined spaces.

The study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants and the study protocol was approved by the ethics committee of the University of Würzburg, Würzburg, Germany.

### Cardiac imaging and spectroscopy

Double-triggered high-field cardiac magnetic resonance imaging (MRI) and ^{1}H-MRS were performed on all participants using a 3 T magnet (MAGNETOM Trio®, Siemens AG Healthcare Sector, Erlangen, Germany) equipped with a 12-channel phased array coil. Participants were scanned in the supine position. All volunteers underwent a fasting period of $\geq 2$ h prior to imaging. No intravenous (i.v.) contrast agent or any other medication was administered to the study cohort.

To facilitate precise voxel placement in the desired cardiac cycle, as well as evaluation of left ventricular (LV) mass and LV function (expressed as ejection fraction) parameters, conventional dynamic steady-state free precession cine images in the short-axis and four-chamber views were acquired prior to cardiac ^{1}H-MRS. Double-triggered cardiac single voxel [1]H-MR spectra (voxel size 6 ml) were obtained from the interventricular septum as described previously,[5] thus avoiding contamination by epicardial fat (Figure 1a, 1b). Respiratory motion
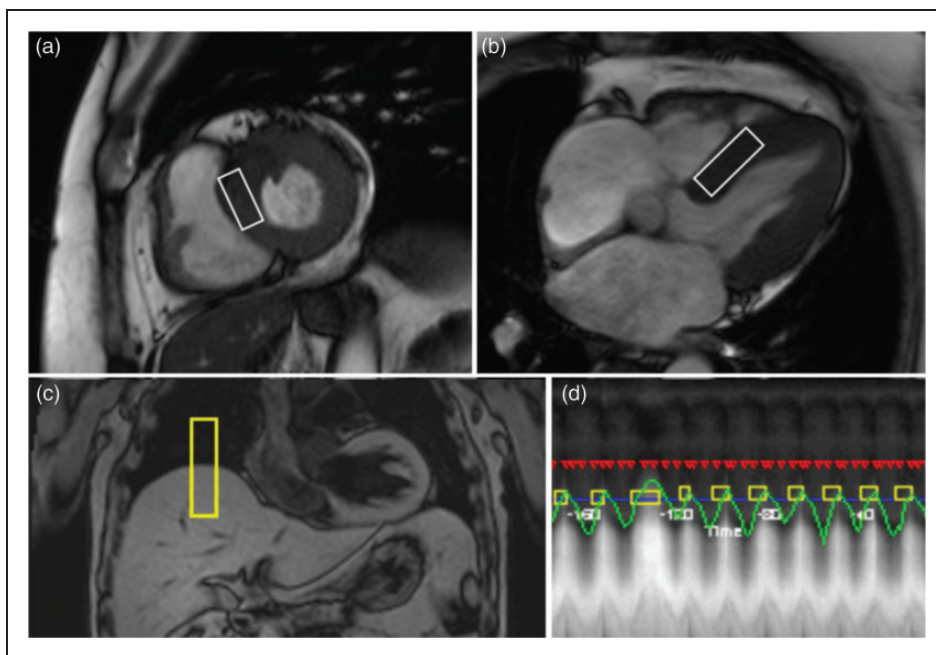
**Figure 1.** Myocardial voxel localization in the interventricular septum for ^{1}H-MRS acquisition, guided by the short-axis (a) and four-chamber (b) views. Respiratory motion gating navigator positioned on the lung-liver interface of the right hemidiaphragma (c) allows unrestricted breathing during spectroscopic data acquisition (d).

gating (navigator positioned on the lung-liver interface of the right hemidiaphragma) allowed unrestricted breathing during spectroscopic data acquisition (Figure 1c, 1d). Single voxel ^{1}H-MRS was performed at end-systole and end-expiration using a point-resolved spectroscopy sequence with an echo time of 35 ms and a repetition time of at least one heartbeat. A set of 32 single data acquisitions was averaged for each spectrum.

## Spectral analysis

Data from ^{1}H-MRS were analysed using commercially available software (Spectroscopy Evaluation®, Siemens AG Healthcare Sector, Erlangen, Germany). For each calculation two spectra (with and without water suppression) were obtained from identical voxel positions. The water signal from the non water-suppressed spectra was used as an internal reference for relative quantification of myocardial triglyceride resonance. Chemical shift of the myocardial triglyceride metabolites on ^{1}H-MRS was measured in parts per million (ppm).

Determination of resonance signals was supported by previous experience at our centre. The water signal was set to a $\partial$-value of 4.7 ppm, consecutively invoking protons in methylene groups to appear at 1.3 ppm, as well as protons in methyl groups at 0.9 ppm. The areas under the curve (AUC) for both triglyceride peaks were summated for the calculation of total myocardial triglyceride content. In accordance with previous studies, relative myocardial triglyceride was calculated by expressing the myocardial metabolite signal as a percentage of the unsuppressed water signal (myocardial triglyceride/water resonance ratio).[5,10–12]

## Statistical analyses

All statistical analyses were performed using statistical software (IBM SPSS® Statistics for Mac, Version 21.0, Armonk, NY, USA). Kolmogorov–Smirnov test was used for evaluation of normal distribution. Mann-Whitney $U$-test was used to determine potential gender differences in myocardial triglyceride content. Two sample $t$-test was used to determine potential gender differences in age, BMI, LV mass and ejection fraction. For myocardial triglyceride content, data were presented as median, interquartile range (IQR) and full range; for age, BMI, LV mass and ejection fraction, data were presented as mean $\pm$ SD. A $P$-value of $< 0.05$ was considered statistically significant.

## Results

In total, 40 healthy volunteers (22 men and 18 women) were included in the study. The characteristics of all participating individuals, assorted according to gender are shown in Table 1. All participants successfully underwent morphological and spectroscopic imaging. As expected, parameters of LV function (i.e., ejection fraction) (men, $63.2 \pm 7.0\%$ [range 50–78%]; women, $65.2 \pm 5.2\%$ [range 56–76%]) and LV mass

(men, $136.3 \pm 25.2$ g [range 70–173 g]; women, $103.9 \pm 16.1$ g [range 79–134 g]) fell within well-established standard values.[7,13]

Both gender groups presented with similar mean $\pm$ SD age and BMI values. The BMI ranged between $18.5$–$33.5$ kg/m$^2$ in men and $18.1$–$31.8$ kg/m$^2$ in women. Comparing male with female volunteers, no gender-specific differences in ejection fraction were observed while on average, men presented with a higher LV mass than women ($p < 0.01$).

## Spectroscopic measurements of myocardial triglyceride content

Myocardial $^1$H-MRS was successfully performed in all participants. Figure 2 shows typical examples of myocardial $^1$H-MR spectra from a 22-year-old man (Figure 2a) and a 24-year-old woman (Figure 2b) with normal BMI values.

The gender-independent averaged myocardial triglyceride values were mean $\pm$ SD, $0.41 \pm 0.47\%$; median 0.25% (IQR 0.16–0.43%). There was no statistically significant difference in myocardial triglyceride content between male (0.28%; IQR 0.17–0.42% [range 0.1–2.3%]) and female volunteers (0.24%; IQR 0.14–0.45% [range 0.1–2.1%]) (Table 1 and Figure 3).

**Table 1.** Characteristics of the healthy volunteers in this study assessing potential gender-related differences in myocardial triglyceride content using $^1$H-Magnetic Resonance Spectroscopy.

| Characteristic | Male volunteers | Female volunteers | Statistical significance |
|---|---|---|---|
| Patients, $n$ | 22 | 18 | |
| Age, mean $\pm$ SD, years | $31.9 \pm 7.9$ | $31.6 \pm 11.0$ | *n.s.* |
| BMI, mean $\pm$ SD, kg/m2 | $22.9 \pm 3.4$ | $21.5 \pm 3.6$ | *n.s.* |
| Ejection fraction, mean $\pm$ SD, % | $63.3 \pm 7.0$ | $65.2 \pm 5.2$ | *n.s.* |
| LV mass, mean $\pm$ SD, g | $136.3 \pm 25.2$ | $103.9 \pm 16.1$ | *P < 0.01* |
| Myocardial triglyceride, median, % | 0.28 | 0.24 | *n.s.* |
| (IQR) | (0.17–0.42) | (0.14–0.45) | |
| (Range) | (0.10–2.30) | (0.10–2.10) | |

SD, standard deviation; BMI, body mass index; LV, left ventricle; IQR, interquartile range; n.s, not statistically significant
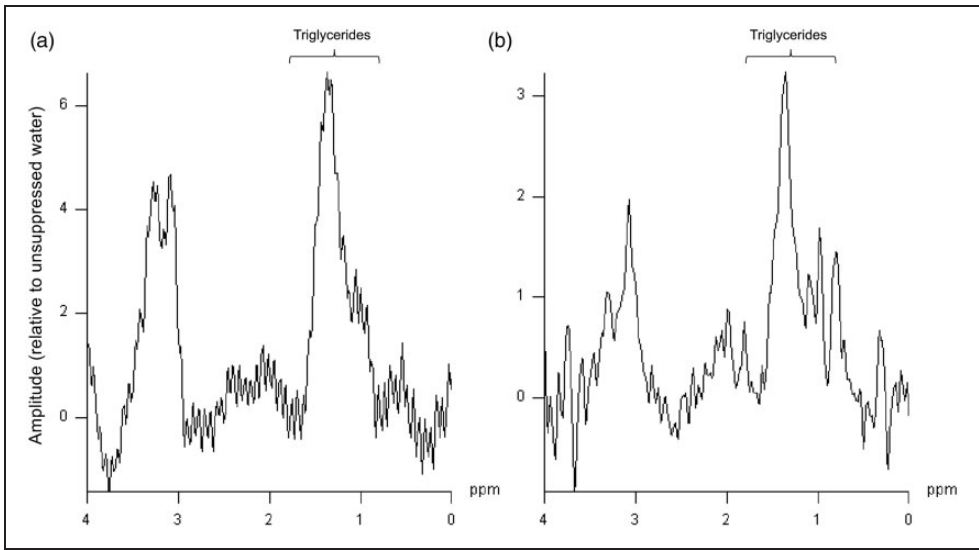
**Figure 2.** Typical examples of proton (hydrogen 1)-magnetic resonance spectroscopy ($^1$H-MRS) from healthy 22-year-old male (a) and 24-year-old female (b) volunteers. Both volunteers showed similar myocardial triglyceride content.
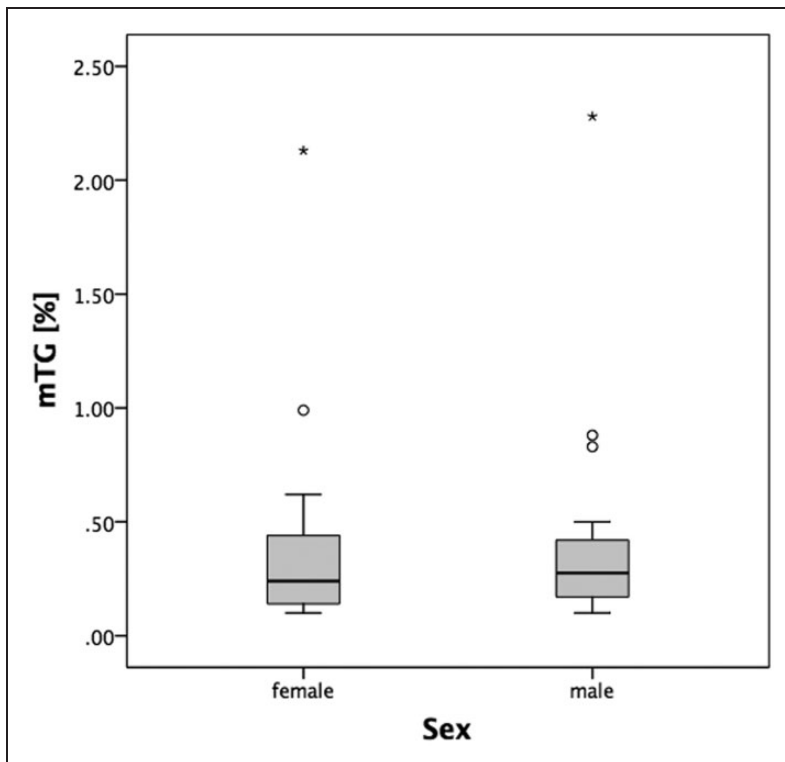


**Figure 3.** Myocardial triglyceride (mTG) content (expressed as mTG/water resonance ratio in %) was similar in both genders.

## Discussion

The present study used ^{1}H-MRS at 3 T to determine potential gender-related differences in the myocardial lipid content in healthy individuals. Normal relative myocardial triglyceride values, as well as concentration ranges, were assessed for both genders. The results from this study showed that myocardial triglyceride concentration was not related to gender.

The voxel size of $20 \times 15 \times 20 \, \mathrm{mm}^3$ enabled an accurate voxel placement in the interventricular septum. As described previously, this voxel position is associated with minimal risk of acquiring false-positive results that might arise from lipid contamination of the voxel by epicardial fat.[5,7] The method itself, as well as the clinical application of ^{1}H-MRS, has made substantial progress throughout the past decade. The benefits of respiratory navigator technique, which significantly improves the reproducibility of *in vivo* myocardial triglyceride determination using a 1.5 T scanner, have been described.[5]

The role and influence of myocardial triglyceride in the human body and especially its impact on heart function have been studied intensively by cardiac ^{1}H-MRS.[7,8,11,12,14] For example, myocardial steatosis was found to be an independent predictor of diastolic function in humans.[14] Moreover, pathological conditions, such as obesity and diabetes mellitus type II, have been shown to be related to myocardial triglyceride content.[8,14] However, few studies have examined the influence of gender on myocardial triglyceride distribution in humans.[9]

Although there were a number of individuals from both sexes with myocardial triglyceride values in excess of 2%, overall, the gender-independent mean myocardial triglyceride values obtained within this study population $(0.41 \pm 0.47\%)$ were in accordance with previous data.[8,10] For example, in one study by McGavock et al., the myocardial triglyceride levels in lean subjects (mean

BMI $23 \, \mathrm{kg/m}^2$) was found to be mean $\pm$ SD $0.46\% \pm 0.30$.[8] However, in another study by a different group, the mean gender-dependent myocardial triglyceride concentrations (i.e., 6.7% in men and 12.3% in women) were much higher than values observed in this present study.[9] Nevertheless, by comparison with the present study which used ^{1}H-MRS at 3 T, the previous study acquired data at a lower field strength of 1.5 T, which may have influenced the measurement of myocardial triglyceride values.[9] Perhaps this could in part be due to a reduced spectral resolution, as well as a lower signal-to-noise ratio at the low field strength.

The present study had some limitations. Although not statistically significant, a minor imbalance in the average BMI was observed in the study population, with a higher mean BMI being observed in the male group. As myocardial triglyceride content is thought to be related to BMI, this may well have influenced the levels in this group. Therefore, by comparison with the findings from this present study, accurately BMI-matched gender cohorts may well show a lower myocardial triglyceride content in male subjects. Further research is required to explore this hypothesis.

Myocardial ^{1}H single voxel spectra were obtained from the interventricular septum only. As a result, data from the present study may not be representative for the entire left ventricle. Furthermore, one must be aware that, following the approach of most former ^{1}H-MRS studies, the analyses of cardiac metabolism within this study are semiquantitative, and rely on the determination of triglyceride/water resonance ratios.[1,2,6] Absolute quantification of myocardial lipid concentration would need myocardial biopsy, which is not without risk. Nevertheless, a high specificity of *in vivo* intramyocardial triglyceride measurement in the human heart has been demonstrated.[6] Finally, please note that individual subjects within this study cohort also serve as healthy

controls in other ${}^1$H-MRS studies undertaken by our group. Consequently, data obtained from study subjects may be evaluated with respect to other issues in additional ${}^1$H-MRS studies.

In conclusion, the study confirmed that ${}^1$H-MRS is a reliable, noninvasive, and sensitive tool for the measurement of myocardial lipids (which have already been established as important biomarkers of myocardial metabolism). Using ${}^1$H-MRS, the results from this study showed that myocardial triglyceride concentration was independent of gender in this group of healthy volunteers. The gender-specific myocardial triglyceride values obtained in this study may be valuable as reference values in future studies evaluating metabolic disorders using magnetic resonance spectroscopy, and also may be useful in routine clinical practice.

## Declaration of conflicting interest

## Funding

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