

# Smoking results in accumulation of ectopic fat in the liver

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**Objective:** An association between smoking and nonalcoholic fatty liver disease has been reported. However, objective quantification of intrahepatic fat via magnetic resonance spectroscopy (MRS) in relation to smoking has rarely been performed in previous studies. Moreover, the possible pathways via which smoking could induce ectopic fat accumulation have not yet been addressed. The current study aimed to examine the association between smoking status and intrahepatic fat quantity and explore the possible mediating effects of triglycerides (TG) and adiponectin.

**Subjects and methods:** Magnetic resonance imager (MRI) spectra were analyzed to quantify intrahepatic fat in 45 men who were on average 62.3 years of age. Smoking status and alcohol intake were self-reported. Accelerometers were used to record daily total physical activity. Fasting blood TG and adiponectin levels were measured enzymatically. Differences in mean intrahepatic fat values according to smoking status were assessed using analysis of covariance.

**Results:** A stepwise increase in mean intrahepatic fat was observed between never, former, and current smokers, respectively, independent of age, physical activity, alcohol intake, and body mass index (BMI) ( $P=0.005$ ). Adjustment for TG and adiponectin significantly attenuated this association ( $P=0.074$ ).

**Conclusion:** Current smoking was significantly associated with increased intrahepatic fat, which may be a result of adipocyte dysfunction, manifested as high circulating TG concentrations and low adiponectin levels.

**Keywords:** intrahepatic fat, cigarette smoking, adiponectin, triglycerides, cross-sectional study

## Introduction

Cigarette smoking is associated with increased risk of diabetes<sup>1</sup> and dyslipidemia.<sup>2</sup> Intrahepatic fat accumulation, a phenomenon in which fat is ectopically deposited in the liver, is also frequently associated with insulin resistance and dyslipidemia.<sup>3</sup> Smoking, which is typically associated with reduced deposition of subcutaneous fat relative to non-smoking,<sup>4</sup> has emerged as a potential risk factor for the progressive accumulation of intrahepatic fat. According to a few observational studies, smoking is associated with increased risk of nonalcoholic fatty liver disease (NAFLD) detected by ultrasonography.<sup>5</sup> However, there is a paucity of data regarding whether smoking is actually related to increased ectopic fat,<sup>6</sup> as one previous study conducted in German found that smoking was not associated with increased liver fat content quantified by magnetic resonance imaging (MRI).<sup>7</sup> Moreover, the possible mediators through which

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smoking could induce ectopic fat accumulation in the liver have not been addressed in previous studies. We speculate that smoking impairs adipocyte function<sup>8–10</sup> and that in the presence of a positive energy balance, triglycerides (TG) efflux leads to adaptive storage in the liver. Indeed, it is well recognized that smoking often decreases adiponectin,<sup>9,10</sup> an adipose-specific adipokine, while increasing circulating TG.<sup>11,12</sup> This may indicate a reduced capacity of adipocytes to deposit fat.

Therefore, we conducted the present study to examine the association between smoking status and intrahepatic fat, using magnetic resonance spectroscopy (MRS), a noninvasive method of measuring lean-tissue lipid content *in vivo*,<sup>3</sup> and to explore the possible mediating effects of TG and adiponectin.

## Methods

### Subjects

As previously reported,<sup>3</sup> civil servant retirees were contacted as potential participants in this study. Prior to recruitment, MRS pretests were conducted on six individuals to estimate the necessary sample size. Based on the resulting mean intrahepatic fat value of 0.105/water signal and standard deviation of 0.068, it was calculated that a sample size of 43 would be required to detect an intrahepatic fat difference of 0.07 with two-sided  $\alpha = 0.05$  and  $\beta = 0.1$ . Taking into account the potential loss of data due to technical or logistical issues, we recruited 47 men. Women were not included in the present study due to their low smoking rate. One of the registered individuals could not complete the liver MR scan, and another had an ungradable liver MRS; these two subjects were thus excluded, leaving 45 men aged 37–69 years as participants in the study.

Written informed consent was obtained after the study aims and procedures had been explained to the participants. The study protocol was approved by the Ethical Review Board of the Nagoya University School of Medicine. In addition, the study was conducted in accordance with the 1964 Declaration of Helsinki and its later amendments.

### Magnetic resonance spectroscopy

Localized single-voxel ( $4 \times 4 \times 4 \text{ cm}^3$ )  $^1\text{H}$  spectra were acquired using a 3-T Siemens Magnetic Resonance Scanner (Siemens Trio; Siemens Healthineers, Erlangen, Germany). All subjects were examined in a

supine position after they had fasted for 8 h. A body surface coil was placed on the subject's abdomen. T1-weighted high-resolution MR images were used to localize the voxel of interest for each participant within the right lobe of the liver. Vascular structures and proximity to subcutaneous fat tissue were avoided in the process of localizing the voxels. The single-voxel spectra were obtained using the stimulated-echo acquisition mode sequence with an echo time of 30 ms and a repetition time of 4000 ms. Shimming was performed automatically, and also manually for water resonance, to optimize the homogeneity of each voxel of interest. Subjects were instructed to hold their breath for about 30 s during the recording of the spectra.

### Intrahepatic fat quantification

Chemical shifts within each spectrum were analyzed using the commercially available software LCModel (version 6.2, Provencher, 1993).<sup>13</sup> The liver lipid estimates determined with LCModel were automatically scaled to the unsuppressed water peak (4.7 ppm). Thus, the values provided by  $^1\text{H}$ -MRS denote the ratio of lipid to water in the volume of interest. In addition, we used the sum of the lipid peaks at 0.9 ppm (methyl) and 1.3 ppm (methylene) relative to water, expressed as a percentage, as indicators of intrahepatic fat content.

### Anthropometric measures and examination of smoking and other lifestyle factors

Body weight and height were measured with the participants wearing scrubs and no shoes. Body mass index (BMI) was calculated as body weight (kg) divided by squared body height ( $\text{m}^2$ ). Smoking status and alcohol consumption were self-reported. Daily alcohol intake (g/day) was calculated by multiplying the weekly frequency and amount for each type of alcohol consumed then dividing this number by seven. Metabolic equivalents (METs) were estimated using an accelerometer (Lifecoder; Suzuken, Nagoya, Japan), which participants were asked to wear for seven days.<sup>14</sup>

### Biochemical assays

Blood samples were stored at  $-80 \text{ }^\circ\text{C}$  prior to performing the biochemical assays. All the assays were carried out at a commercial laboratory using standard procedures. TG levels were determined enzymatically. Adiponectin

concentration was determined via enzyme-linked immunosorbent assay (ELISA; Otsuka Pharmaceutical, Tokyo, Japan).

## Statistical analysis

Intrahepatic fat, TG, adiponectin, and alcohol intake were log-transformed for analysis. Analysis of variance was used to compare baseline characteristics between the smoking-status groups. The differences in mean intrahepatic fat values between smoking-status group were tested using analysis of covariance adjusted for age, log-alcohol intake, and physical activity (Model 1). Further adjustment for BMI was made in Model 2 and for BMI, log-TG, and log-adiponectin in a mediation model. Since the number of current smokers was limited, it was combined with that of former smokers to create an ever smoker group for supplementary analyses. The difference in mean intrahepatic fat values between ever and never smokers was examined. All the analyses were performed with using SPSS

software, version 24.0 (International Business Machines, Armonk, NY, USA).

## Results

The current, former, and never smoker groups comprised 7, 16, and 22 individuals, respectively. Except for TG values, which were higher in current smokers and lower in never smokers ( $P=0.01$ ), there was no significant associations between the covariates we analyzed and smoking status (age, alcohol intake, physical activity, BMI, or adiponectin level; Table 1). In Model 1, the mean intrahepatic fat values were significantly higher in current smokers, independent of age, physical activity, and alcohol intake ( $P=0.005$ ). Further adjustment for BMI in Model 2 weakened the association slightly ( $P=0.007$ ). However, additional adjustment for TG and adiponectin significantly attenuated the association in the mediation model ( $P=0.074$ ; Table 2). The analysis in which ever smokers were compared with never smokers yielded similar results. Specifically, mean intrahepatic fat

**Table 1** Means (standard deviations) and geometric means (95% confidence intervals) according to smoking status

|                                      | Current smokers     | Former smokers     | Never smokers     | P    |
|--------------------------------------|---------------------|--------------------|-------------------|------|
|                                      | (n=7)               | (n=16)             | (n=22)            |      |
| Age (years)                          | 58.6 (7.7)          | 63.8 (2.8)         | 62.7 (6.9)        | 0.11 |
| Alcohol intake (g/day)               | 0.5 (0.02–11.1)     | 3.3 (0.4–27.3)     | 3.2 (0.5–19.5)    | 0.55 |
| Physical activity (METs)             | 2075.9 (179.6)      | 2014.6 (129.1)     | 1998.0 (244.7)    | 0.76 |
| Body mass index (kg/m <sup>2</sup> ) | 26.1 (5.4)          | 24.0 (2.5)         | 23.9 (2.0)        | 0.31 |
| Triglycerides levels (mg/dl)         | 182.9 (122.9–272.1) | 104.4 (80.3–135.8) | 94.7 (75.6–118.5) | 0.02 |
| Adiponectin levels (µg/ml)           | 6.6 (5.0–8.0)       | 7.1 (5.9–8.6)      | 8.7 (7.4–10.2)    | 0.16 |

**Note:** Differences were assessed using ANOVA.

**Table 2** Log-intrahepatic fat (95% confidence intervals) according to smoking status in men (n=45)

|                 | Current smokers        | Former smokers         | Never smokers          | P     |
|-----------------|------------------------|------------------------|------------------------|-------|
|                 | Ever smokers           |                        |                        |       |
| Crude           | -0.80 (-1.12 to -0.48) | -1.25 (-1.46 to -1.04) | -1.39 (-1.57 to -1.21) | 0.009 |
|                 | -1.11 (-1.30 to -0.93) |                        | -1.39 (-1.58 to -1.20) | 0.040 |
| Model 1         | -0.75 (-1.08 to -0.41) | -1.26 (-1.47 to -1.05) | -1.40 (-1.58 to -1.22) | 0.005 |
|                 | -1.11 (-1.29 to -0.92) |                        | -1.40 (-1.58 to -1.21) | 0.034 |
| Model 2         | -0.80 (-1.10 to -0.51) | -1.25 (-1.44 to -1.07) | -1.39 (-1.54 to -1.23) | 0.005 |
|                 | -1.12 (-1.28 to -0.96) |                        | -1.38 (-1.55 to -1.21) | 0.031 |
| Mediation model | -0.94 (-1.22 to -0.66) | -1.28 (-1.45 to -1.11) | -1.32 (-1.47 to -1.18) | 0.074 |
|                 | -1.19 (-1.34 to -1.04) |                        | -1.31 (-1.46 to -1.16) | 0.271 |

**Notes:** Model 1 includes age, log-alcohol intake, and physical activity (METs per day). Model 2 includes body mass index and the variables in Model 1. The mediation model includes log-triglycerides, log-adiponectin and the variables in Model 2.

was significantly higher in ever smokers in both Model 1 ( $P=0.034$ ) and Model 2 ( $P=0.031$ ), and further adjustment for blood TG and adiponectin values significantly attenuated the association ( $P=0.271$ ; Table 2).

## Discussion

Current smoking was significantly associated with higher intrahepatic fat levels. This result was consistent with some previous studies,<sup>5</sup> which found that smoking was significantly associated with NAFLD, independent of age, alcohol intake, physical activity,<sup>15</sup> and indicators of body fat such as BMI.<sup>16–19</sup> However, it was inconsistent with another study, which found that smoking was not associated with liver-fat content as quantified by MRI.<sup>7</sup> The reason for this discrepancy is unknown; however, it may be related to differences in the age or ethnicity of the study population.<sup>20</sup> Although we adjusted for BMI while examining the association between smoking and intrahepatic fat, it is possible that some residual confounding by obesity remained.<sup>21</sup> We conducted additional analyses to further adjust for waist circumference, body fat percentage, and total energy consumption in addition to the variables in Model 2, but the significant association between current smoking and intrahepatic fat remained ( $P=0.007$ ). In another supplementary analysis in which the participants were stratified by BMI at 23 kg/m<sup>2</sup>, ever or current smoking appeared to be associated with intrahepatic fat; however, the small sample size precluded any definitive statement (data not shown).

As we postulated, TG and adiponectin levels together explained the significant association between smoking and intrahepatic fat. In other words, the effect of smoking on intrahepatic fat accumulation may be mediated by a state characterized by low adiponectin and high TG, although the cross-sectional nature of the present study prohibits us from making causal inferences. Nevertheless, one of the possible mechanisms for the association between smoking and intrahepatic fat could be that current smokers with excess energy intake may need to store surplus TG ectopically in the liver as a result of smoking-related adipocyte dysfunction, a state presumably represented by decreased adiponectin.<sup>10,22</sup> This speculation is supported by a report that the adiponectin rs266729 polymorphism (G allele), which is related to reduced adiponectin, is associated with NAFLD, particularly in smokers.<sup>17</sup>

Based on our observation that adjustment for blood TG and adiponectin levels attenuated the significant association

between smoking and liver fat accumulation, we speculate that high TG and low adiponectin levels may exist prior to the accumulation of liver fat, since this is reportedly a sign of impaired adipocyte capacity, which leads to adaptive TG storage in tissues other than adipocytes.<sup>11,12</sup> Indeed, HIV-infected patients receiving antiretroviral treatment often exhibit low subcutaneous fat and elevated circulating TG levels.<sup>23</sup> Similarly, a clinical study of burn patients showed that the peripheral release of fatty acids, rather than endogenous hepatic fat synthesis, is causally related to hepatic lipid accumulation.<sup>24</sup>

On the other hand, insulin resistance coexists with increased TG, decreased adiponectin levels, and NAFLD. We therefore postulate that hepatic insulin resistance (high fasting insulin levels) could be a consequence of liver fat accumulation, rather than of abnormal TG and adiponectin levels.<sup>25</sup> In support of this idea, a recent 9-year follow-up study confirmed that higher baseline TG content was significantly associated with the development of NAFLD.<sup>26</sup> In order to investigate this idea epidemiologically, we performed an additional mediation analysis adjusting for insulin resistance index instead of TG and adiponectin. In this analysis the significant association between smoking and intrahepatic fat remained unchanged or became somewhat stronger (data not shown). This was consistent with a previous study, which reported that adjustment for fasting serum insulin strengthened the positive association between smoking and NAFLD.<sup>16</sup>

The present study has several limitations. First, the cross-sectional design of the study restricted our ability to make causal inferences. Our results should therefore be confirmed in prospective or interventional studies. Second, the number of participants was small. Even though the sample size was determined *a priori*, some of the statistically nonsignificant findings might have been so due to the small sample size. The small sample size also precluded the use of additional confounding variables to further adjust the model. Finally, the present study was conducted on retirees from a local government office in Japan, so caution should be taken in generalizing these findings, and further studies in other settings are warranted.

In conclusion, current and ever smoking status was significantly associated with increased intrahepatic fat, which may be explained by adipocyte dysfunction manifested as high circulating TG concentrations and low adiponectin levels.

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## Disclosure

The authors report no conflicts of interest in this work.

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