

# ***Positive association between blood C3 level and liver fat content quantified by 1H magnetic resonance spectroscopy in Japanese men***

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# ***Introduction***

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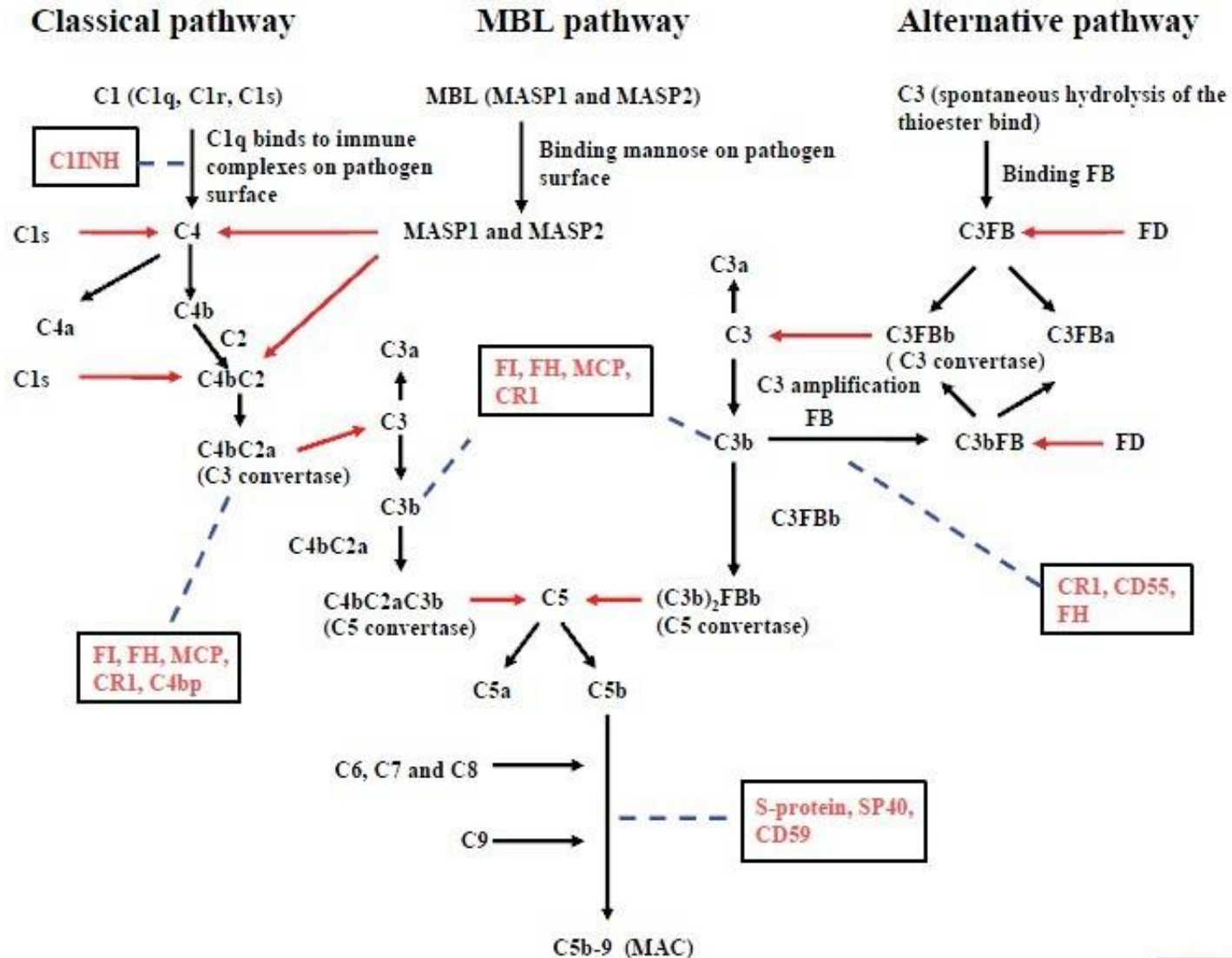
■ Hepatosteatorosis, or fatty liver disease, occurs through the abnormal retention of lipids within a hepatocyte.

✓ The complement pathway is an important component of the innate and adaptive immune response, which includes classic, lectin, or alternative pathways. All of these 3 pathways culminate in the activation of C3.

✓ It has been suggested that the development of hepatosteatorosis requires C3.[1] Neutrophil infiltration into tissues is strongly promoted by C3a and C5a, factors that are generated by intermediate cleavage steps in the complement cascade.

[1]Pritchard, M. T., M. R. McMullen, et al. (2007). "Differential contributions of C3, C5, and decay-accelerating factor to ethanol-induced fatty liver in mice." Gastroenterology 132(3): 1117-1126.

# Figure 1 : 3 pathways of complement system



[2] Qin X, Gao B. The complement system in liver diseases. Cell Mol Immunol 2006;3:333-40.

# ***Introduction***

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- ✓ Activation of the complement system has previously been implicated in the pathogenesis of alcoholic steatohepatitis. C3-deficient mice display attenuated hepatic steatosis after ethanol challenge.
- ✓ Moreover, several components of the complement system are dysregulated in obesity and insulin resistance, which are both associated with NAFLD.

## ***Aim***

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- ✓ We aimed to determine whether in healthy Japanese men there will be a relationship between the serum C3 and MRS quantified liver fat content.

## **Methods: Subjects and blood test**

- ✓ Subjects were retirees of a local government recruited by a letter (n = 55, 85.5% men) and were fasting eight hours or more at the time of examination.
- ✓ We restricted present analysis to male participants who drank less than 46g alcohol per day without missing in necessary variables (n = 40).
- ✓ Serum samples were assessed for levels of C3, insulin, aspartate transaminase (AST), alanine transaminase (ALT), and other general markers of inflammation (C-reactive protein [CRP], tumor necrosis factor [TNF]- $\alpha$  and interleukin [IL]-6).

## ***Methods: MRS***

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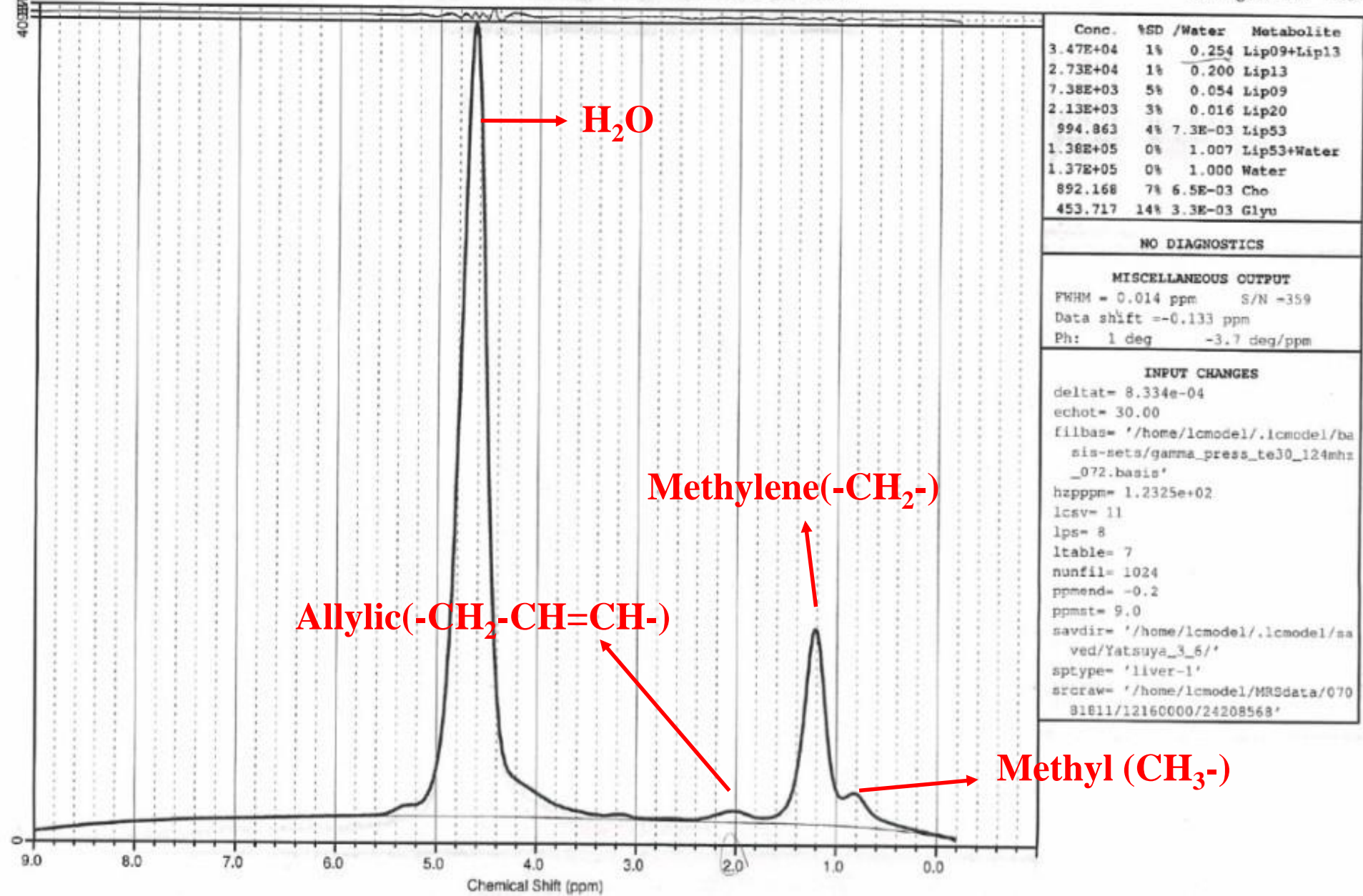
- MRS is a reliable and non-invasive method to quantify liver fat content.
- ✓  $^1\text{H}$  magnetic resonance spectroscopy ( $^1\text{H}$ -MRS)
- ✓ 3T Siemens Magnetic Resonance Scanner (TE=30ms)
- ✓ Volume of interest: right liver lobe (4×4×4 cm)
- ✓ Spectrogram was analyzed by LCModel software
- ✓ Major lipid peaks at 0.9ppm representing methyl protons (-CH<sub>3</sub>) and 1.3ppm representing methylene protons (-CH<sub>2</sub>-) were used as an indicator of liver fat content.

# Figure 2: An example of spectrogram

LCModel (Version 6.1-4G) Copyright: S.W. Provencher.

Ref.: Magn. Reson. Med. 30:672-679 (1993).

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## ***Statistical analysis***

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- Subjects were categorized by tertiles of total liver fat content, and labeled as low, medium and high.
- Age, body mass index, smoking status, and alcohol intake were used as covariates



# *Subject characteristics:*

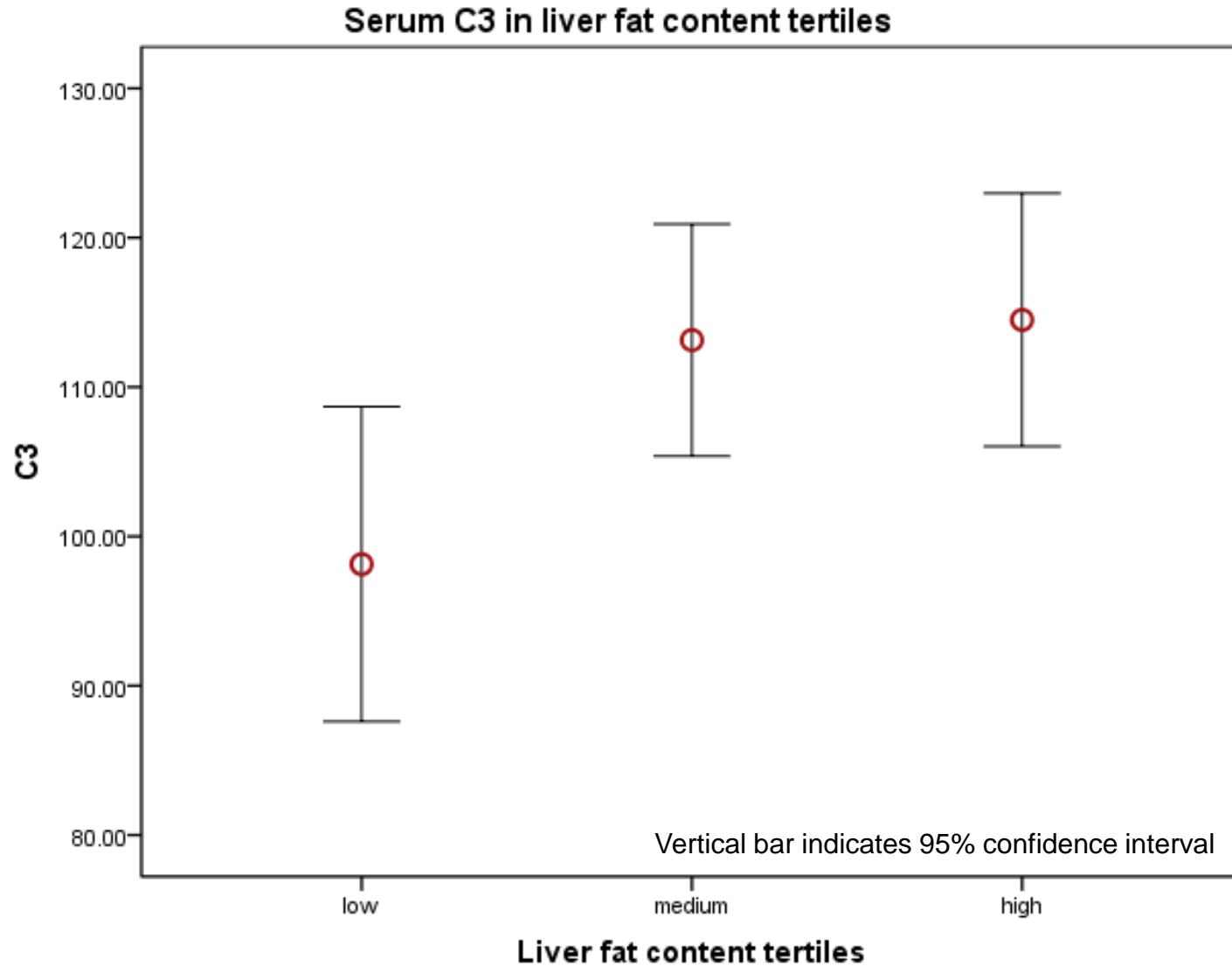
	Liver fat content tertiles				p
	Total	Low	Medium	High	
Liver fat (logLip09_13PerWater)	-1.3±0.5	-1.7±0.2	-1.2±0.2	-0.7±0.3	
Age	62.5±6.4	64.1±2.6	60.4±7.7	62.5±7.9	0.326
BMI	24.5±3.2	23.1±1.7	24.0±2.1	26.0±3.9	0.029
Alcohol intake	15.5±14.6	14.4±16.4	16.2±13.7	15.5±15.9	0.954
Tobacco (current, past, never)	6, 13, 21	1, 4, 9	1, 4, 9	4, 5, 3	

# ***Results: Multivariate-adjusted means of C3 according to liver fat content tertile***

	liver fat content groups				
	low	medium	high	p	linear p
logC3	4.57 ± 0.04	4.71 ± 0.04	4.72 ± 0.05	0.024*	0.029*
logCRP	5.81 ± 0.35	6.45 ± 0.31	6.08 ± 0.37	0.371	0.623
logTNF- $\alpha$	0.09 ± 0.23	0.19 ± 0.20	0.13 ± 0.24	0.951	0.913
logIL-6	0.01 ± 0.12	0.21 ± 0.11	0.34 ± 0.13	0.233	0.106
logAST	3.10 ± 0.08	3.12 ± 0.08	3.20 ± 0.09	0.747	0.464
logALT	3.09 ± 0.15	3.10 ± 0.14	3.16 ± 0.16	0.950	0.769
logInsulin	1.13 ± 0.18	1.71 ± 0.16	1.64 ± 0.19	0.048*	0.079

***Figure 3: Serum level of C3 increases with the accumulation of liver fat content***

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## **Discussion**

- ✓ Because C3 indicates clearance of apoptotic cells and promotion of liver regeneration, it is possible to speculate that there exists tissue injury inside the liver.
- ✓ Accumulation of triglycerides (TG) in the liver, independently of the initial cause, leads to lipotoxicity, generation of oxidative stress and inflammation. Our study may suggest that liver injury may begin as long as the lipid inside liver tissue increases, even without diagnosis of fatty liver disease.

# ***Discussion***

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✓ One of the breakdown products of C3, termed acylation-stimulating protein (ASP) possibly plays a key role that since it promotes triglycerides accumulation in hepatocytes, thereby creating a vicious cycle that complement activation promotes hepatosteatosis, and that further increases complement activation.[3]

[3] Valenti L, Fracanzani AL, Fargion S. The immunopathogenesis of alcoholic and nonalcoholic steatohepatitis: two triggers for one disease? *Semin Immunopathol* 2009;31:359-69.

# ***Discussion***

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✓ Although the evolutionary purpose of immunity is to defend against pathogens and foreign substances, in the setting of obesity, dietary fatty acids, especially oxidized fatty acids may be perceived as foreign substances that modulate inflammation. And the activation of immune pathways can adversely affect hepatic lipid metabolism leading to hepatic injury, steatohepatitis, and fibrosis.

## **Conclusion**

- ✓ The complement system and especially its component C3 appear to be associated with the accumulation of lipid content within the liver tissue.
- ✓ Clarifying the mechanisms whereby C3 contributes to hepatosteatosis may ultimately help in understanding the pathogenesis of fatty liver disease.