TNF-α is not the cause of fatty liver disease in obese diabetic mice

To the editor—The article by Lin *et al.*[1] in the September 2000 issue of *Nature Medicine* proposed that metformin treatment reverses fatty liver disease (FLD) in obese, leptin-deficient (ob/ob) mice by decreasing hepatic TNF-α mRNA expression. In that study, metformin reversed hepatomegaly, lowered elevated serum transaminase levels, and decreased TNF-α, uncoupling protein 2 (UCP2) mRNA and fatty acid synthase protein in the liver in ob/ob mice. Because earlier studies have shown that TNF-α is elevated in ob/ob mice, stimulates hepatic fatty acid synthesis and induces UCP2 expression in the liver, the authors postulated that TNF-α and TNF-inducible factors that promote hepatic lipid accumulation contribute to FLD in obese mice.

While the hypothesis that increased hepatic TNF-α expression caused FLD is attractive, it is also conceivable that the increase in hepatic TNF-α expression is a consequence of lipid accumulation rather than the cause. One approach to obtain definitive evidence for or against the role of TNF-α as a mechanism of FLD is to use knockout mice. Previous studies have shown that ob/ob mice that lack both TNF receptors (ob/ob–p55/p75-null) and therefore lack functional TNF-α activity, have improved insulin sensitivity[2], but no change in body weight or fat mass per lean body mass[3]. We have reported that ob/ob–p55/p75-null mice have twice as much UCP2 expression in the liver and adipose tissue compared with ob/ob mice[4] suggesting that endogenous TNF-α has an inhibitory effect on UCP2 and therefore a lack of TNF-α further upregulates UCP2 expression in obese mice. Since these data were in contrast to the proposed hypothesis of Lin *et al.*[1] that TNF-α may be the cause of FLD and subsequent susceptibility to liver toxicity in ob/ob mice, we have measured hepatic lipid content and serum transaminase levels in control, ob/ob, p55/p75-null and ob/ob–p55/p75-null mice.

We show that hepatic lipid content is similar in ob/ob and ob/ob–p55/p75-null mice (Table 1). Moreover, serum transaminase levels are also not significantly different between ob/ob mice with or without TNF receptors. Thus, these data demonstrate that lack of TNF-α action neither reverses hepatic lipid accumulation nor lowers elevated serum transaminase levels in obese mice, indicating that TNF-α is not the mechanism of FLD in obese diabetic mice. Our data indicate that FLD occurs in ob/ob mice, even in the absence of TNF action and TNF-induced insulin resistance.

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Table 1 Total hepatic lipid content and serum transaminase levels in ob/ob and ob/ob–p55/p75-null mice.

Experimental Group	Hepatic Lipid Content (mg/g wet weight)	Serum Alanine Aminotranferase (U/L)	Serum Aspartate Aminotransferase (U/L)
Control mice	29.2 ± 1.2	13.6 ± 0.6	19.9 ± 3.4
ob/ob mice	59.1 ± 1.4	46.4 ± 4.2	35.5 ± 6.1
P vs. control	< 0.001	< 0.001	NS
p55/p75-null mice	34.1 ± 1.3	14.8 ± 4.3	19.7 ± 3.6
P vs. control	< 0.05	NS	NS
ob/ob–p55/p75-null mice	57.6 ± 3.1	47.6 ± 12.8	44.1 ± 11.9
P vs. p55/p75-null mice	< 0.001	< 0.05	NS

Age matched control, ob/ob, p55/p75-null and ob/ob–p55/p75-null mice (8-wk-old male mice, $n = 5$ in each group) were maintained on chow and water *ad libitum.* After 1 wk mice were killed; total hepatic lipid content and serum transaminase levels measured by standard methods. Data are presented as mean ± s.e.m; analysis of variance was used to determine statistical significance. All mice gifts of G.S. Hotamisligil (Harvard University, Boston, Massachussetts}.

Diehl replies—Memon and colleagues report that hepatic lipids and serum aminotransferases are similar in ob/ob wild-type mice and ob/ob mice that are genetically deficient in both TNF receptors, apparently refuting our hypothesis that increases in hepatic TNF-α contribute to the pathogenesis of FLD. Recently, they also noted increased UCP-2 mRNA levels in the livers of TNF-α receptor-deficient ob/ob mice[4], casting further doubt on our suggestion that TNF-related induction UCP-2 compromises hepatocyte viability in fatty livers. It is important, however, to emphasize that several issues remain unresolved.

Serum aminotransferase values were normal in both strains of ob/ob mice that Memon and colleagues studied. We find, however, that serum aspartate aminotransferase (AST) and alanine aminotransfarese (ALT) values in adult male ob/ob mice are significantly higher than in age- and gender-matched litter mates[1,6]. These differences might be important because FLD begins with fatty liver (steatosis) and progresses to fatty liver with inflammation and liver cell injury (steatohepatitis). The release of liver-derived aminotransferases into the serum occurs predominately during steatohepatitis[7]. Thus, the ob/ob mice that we studied had steatohepatitis, while Memon's ob/ob mice were probably at the earlier, steatotic stage. Another group has reported that TNF-receptor–deficient mice are completely protected from steatohepatitis induced by alcohol, demonstrating the importance of TNF-α at the inflammatory stage of fatty liver disease[8].

Moreover, alcohol- or obesity-induced fatty livers are unusually vulnerable to TNF-α–mediated injury[6,8]. This vulnerability results partly from hepatocyte mitochondrial adaptations to chronic oxidative stress[9,10]. We proposed that activation of mitochondrial uncoupling proteins might be involved in this process after noting that UCP-2 mRNA, protein and activity are increased in ob/ob fatty hepatocytes but repressed in ob/ob macrophages[11]. This pattern of UCP-2 expression differs from normal livers, where UCP-2 is localized predominantely in macrophages and barely expressed in hepatocytes[12]. Because TNF-α upregulates