Tumor Necrosis Factor System Activity Is Associated With Insulin Resistance and Dyslipidemia in Myotonic Dystrophy

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Myotonic dystrophy (MyD) is a multisystem autosomal dominant disorder associated with progressive muscle wasting and weakness. The striking metabolic abnormality in MyD is insulin resistance. The mechanism by which target tissues are insensitive to insulin action remains uncertain. In a recent study, plasma soluble tumor necrosis factor receptor (sTNFR)2 levels were found to be associated with muscle tissue mass and insulin resistance. Given these associations, we speculated that disorders of the muscle cell membrane could lead simultaneously to insulin insensitivity and sTNFR2 leakage in MyD. To test this hypothesis, we measured the levels of circulating sTNFR1 and sTNFR2 and insulin resistance in MyD patients. We studied 22 MyD patients and 24 age-, BMI-, and fat mass–matched control subjects. Both MyD men and women showed higher plasma insulin levels in the presence of comparable glucose concentrations than did control subjects. sTNFR2, but not sTNFR1, levels were ~1.5-fold higher in MyD patients. In parallel with these findings, the fasting insulin resistance index (FIRI) was also higher in MyD patients. In fact, in the whole population, fasting insulin and FIRI strongly correlated with sTNFR2 in both men ($r = 0.77$ and $r = 0.81$, $P < 0.0001$, respectively) and women ($r = 0.67$ and $r = 0.64$, $P = 0.001$, respectively). sTNFR2 levels were also associated with the insulin sensitivity index (S_I), calculated from an oral glucose tolerance test (OGTT) according to the method by Cederholm and Wibell ($r = -0.43$, $P = 0.006$). We constructed a multiple linear regression to predict FIRI, with BMI, waist-to-hip ratio, and sTNFR2 as independent variables. In this model, both BMI ($P = 0.0014$) and sTNFR2 ($P = 0.0048$) levels contributed independently to 46% of the variance of FIRI. In another model, in which FIRI was substituted for S_I from the OGTT, both BMI ($P = 0.0001$) and sTNFR2 ($P = 0.04$) levels contributed independently to 48% of the variance of S_I from the OGTT. Plasma cholesterol and triglyceride concentrations were significantly increased in MyD patients. sTNFR1 and sTNFR2 levels were found to be strongly associated with plasma cholesterol, LDL cholesterol, and triglycerides. sTNFR1 and sTNFR2 also correlated with serum creatine kinase activity in MyD patients ($r = 0.57$, $P = 0.006$; $r = 0.75$, $P < 0.0001$, respectively). In conclusion, here we describe, for the first time to our knowledge, a relationship between insulin action and plasma sTNFR2 concentration in MyD patients. We have also found increased concentrations of plasma triglycerides and cholesterol levels in parallel with sTNFR1 and sTNFR2 concentrations in MyD patients. We speculate that the latter associations are dependent on, and secondary to, increased tumor necrosis factor (TNF)-α action. Whether TNF action is implicated in the pathogenesis of MyD or is a simple marker of disease activity awaits further studies. *Diabetes* 48:1108–1112, 1999

Myotonic dystrophy (MyD) is a multisystem autosomal dominant disorder associated with progressive muscle wasting and weakness. The onset of symptoms typically occurs in the 2nd and 3rd decades of life. MyD is the most common adult form of muscular dystrophy, with an estimated prevalence of 1 in 8,000 (1). The MyD gene has been identified as an expansion mutation of a trinucleotide repeat at chromosome region 19q 13.3 (2). The gene product is a protein kinase (PK) of metabolic importance to muscle.

The striking metabolic abnormality in MyD is insulin resistance. Marked hyperinsulinemia after oral glucose, intravenous glucose, tolbutamide, glucagon, or arginine tolerance tests is observed in MyD patients (3,4). The mechanism by which target tissues are insensitive to insulin action remains uncertain, although a receptor or postreceptor defect has been suggested (3,4).

In recent years, it has been demonstrated that tumor necrosis factor (TNF)-α has important effects on whole-body lipid and glucose metabolism (5,6). TNF-α is a candidate mediator of insulin resistance because it is overexpressed in the adipose and muscle tissues of rodents and humans and

blocks the action of insulin in cultured cells and whole animals (7–9). The induction of insulin resistance is mediated through TNF-α's ability to produce serine phosphorylation of insulin receptor substrate-1, decreasing the tyrosine kinase activity of the insulin receptor (10).

TNF signals through at least two known cell surface receptors (TNFRs), TNFR1 (p55) and TNFR2 (p75), that are present in virtually all cells of higher mammals (11–13). It appears that TNFR1 can signal for virtually all known activities of TNF, including apoptosis, differentiation, and proliferation. TNFR2 seems to signal metabolic actions (13). Both receptors for TNF exist also in soluble forms (14,15), apparently derived by proteolytic cleavage from the cell surface forms (16). These soluble TNFRs (sTNFRs) can compete with the cell surface receptors and thus block their activity. However, sTNFRs affect TNF function also by stabilizing TNF activity, most likely by preventing dissociation of the homotrimeric TNF molecules to inactive monomers (16). It has been suggested that sTNFR1 and sTNFR2 represent markers of previous TNF-α activity by forming a "slow release reservoir" and impeding spontaneous denaturation of the cytokine, remaining elevated for several days after TNF-α administration (16).

In a recent study, obese women expressed approximately twofold more TNFR2 mRNA in fat tissue and approximately sixfold more sTNFR2 in circulation relative to lean control subjects (17). Adipose tissue TNFR2 expression strongly correlated with BMI and the level of insulinemia. We have recently found that plasma sTNFR2 levels were inversely associated with insulin sensitivity in healthy subjects (18). Furthermore, sTNFR2 levels correlated positively with parameters of the muscle compartment, such as fat-free mass, mid-arm muscle circumference, and mid-arm muscle area (18). In contrast, no correlation was observed between any metabolic variable and adipose tissue TNFR1 expression (17) or plasma sTNFR1 levels (18).

Muscle fibers displayed enhanced expression of TNF-α, and upregulation of cytokines was strongest at sites of cellular infiltration, typical for the respective myositis subtype in two recent studies dealing with inflammatory myopathies (19,20). Unfortunately, MyD patients were not included in these reports. We are unaware of any study investigating TNF expression or TNF action in MyD. Given the associations between sTNFR2 levels and the muscle compartment, we speculated that disorders of the muscle cell membrane could lead simultaneously to insulin insensitivity and sTNFR2 leakage. To test this hypothesis, we measured the levels of circulating sTNFR1 and sTNFR2 and insulin resistance in MyD patients.

RESEARCH DESIGN AND METHODS

Subjects. We studied 22 MyD patients and 24 control subjects who were similar in age, BMI, and fat mass (Table 1). Characteristics of the subjects are summarized in Table 1. MyD diagnosis was based on clinical measures and on evidence of CTG repeats in the 3′-untranslated region of the gene involved in MyD pathogenesis. Inclusion criteria for patients and subjects included *1*) BMI (weight in kilograms divided by the square of height in meters) < 40 kg/m^2; *2*) absence of any systemic disease other than MyD; and *3*) absence of any infections in the previous month. None of the subjects were taking any medication known to interfere with glucose or lipid metabolism or had any evidence of metabolic disease other than obesity. All reported that their body weight had been stable for at least 3 months before the study. Metabolic and hormonal data on some of the subjects were reported previously (18). The protocol was approved by the hospital ethics committee, and informed consent was obtained from each subject.

Anthropometric measurement. All subjects were evaluated, in addition to BMI, through the waist-to-hip ratio (WHR), as previously described (18). The percentage of body fat and fat-free mass were measured by bioelectric impedance analysis (Holtain BC Analyser, London, U.K.).

Study protocol. An oral glucose tolerance test (OGTT) was performed according to the recommendations of the National Diabetes Data Group (21). After a 12-h overnight fast, glucose was ingested in a dose of 75 g, and blood samples were collected through a venous catheter from an antecubital vein at 0, 30, 60, 90, and 120 min for measurement of serum glucose. The glucose (mean blood glucose [MBG]) and insulin (mean serum insulin [MSI]) total areas under the curve during the OGTT were determined by the trapezoidal method. The degree of peripheral sensitivity to insulin was estimated during the OGTT according to the method validated by Cederholm and Wibell (22). In this method, as in the minimal model developed by Bergman et al. (23), the glucose-insulin homeostatic system is considered to be divided into two parts, pancreatic responsiveness and peripheral cell responsiveness, permitting analysis of each part by input and output observations without disrupting the glucose-insulin feedback relationship (22). The glucose uptake rate in peripheral target tissues (*M*) is expressed as milligrams per minute and is calculated according to the following formula: $M = OGL/120 + [(G_0 - G_{120}) \times 0.19 \times BW]/120$, where OGL is the oral glucose load (75,000 mg), which was used as a constant factor; $G_0 - G_{120}$ is the amount of glucose at the fasting level minus the amount at 120 min, representing the part of the OGL left in the glucose space after 2 h; and BW is the body weight in kilograms. The factor 120 converted 2 h into minutes. When the glucose uptake rate is evaluated during clamp tests, it is usually expressed as the metabolic clearance rate (MCR) to avoid the influence of different blood glucose levels on the uptake rate. For the purpose of assessing the insulin sensitivity index

TABLE 1
Anthropometric and biochemical variables

|	MyD men	Control men	*P* value	MyD women	Control women	*P* value
n	8	11	—	14	13	—
Age (years)	38.5 ± 9.6	39.1 ± 6.7	NS	38.7 ± 5.9	42.3 ± 10	NS
BMI (kg/m^2)	26.8 ± 2.2	26.9 ± 3.7	NS	32.9 ± 2.2	32.5 ± 2.7	NS
WHR	0.98 ± 0.2	0.94 ± 0.3	NS	0.89 ± 0.3	0.91 ± 0.4	NS
Fat mass (kg)	23.1 ± 9.6	19.1 ± 9.57	NS	22.2 ± 10	26.9 ± 12.4	NS
Percent fat mass (%)	30.6 ± 12.3	21.9 ± 9.5	NS	28.6 ± 10.2	33.5 ± 11.1	NS
sTNFR1 (ng/ml)	2.1 ± 0.8	1.61 ± 0.14	NS	1.81 ± 0.7	1.45 ± 0.26	0.08
sNTFR2 (ng/ml)	4.51 ± 1.6	3.06 ± 0.2	0.04	3.91 ± 1.17	2.62 ± 0.28	0.001
Fasting glucose (mmol/l)	5.27 ± 0.49	5.45 ± 0.53	NS	5.41 ± 0.9	5.38 ± 1.3	NS
Fasting insulin (mU/l)	15.4 ± 8	8.7 ± 3.1	0.021	20.7 ± 11	9.9 ± 5.4	0.003
FIRI	3.2 ± 1.34	1.94 ± 0.86	0.031	4.7 ± 2.5	2.16 ± 1.3	0.003
Cholesterol (mmol/l)	5.91 ± 0.8	5.24 ± 0.75	0.08	5.6 ± 0.8	5.25 ± 0.8	NS
LDL cholesterol (mmol)	4.6 ± 0.6	3.8 ± 0.7	0.05	4.3 ± 0.5	3.6 ± 0.8	0.019
Triglycerides (mmol/l)	2.03 ± 0.4	1.19 ± 0.4	0.001	2.41 ± 0.37	1.33 ± 0.8	0.001

Data are means ± SD or *n*.
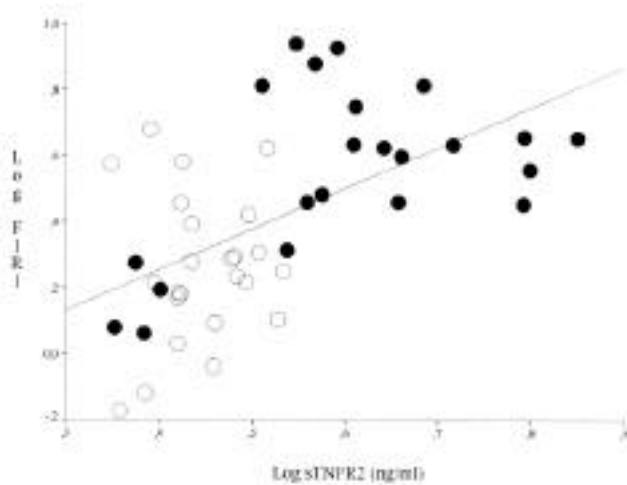
FIG. 1. Linear correlation between circulating sTNFR2 levels and FIRI ($r = 0.57$, $P < 0.0001$). ●, MyD patients.

FIG. 2. Linear correlation between circulating sTNFR2 levels and S_I obtained from the OGTT in all subjects ($r = -0.43$, $P = 0.006$, $n = 46$). ●, MyD patients.

(S_I), the MCR during the OGTT was estimated by correcting the M value with MBG during the 2-h test (MCR [mg · l^{-1} · mmol · l^{-1} · min^{-1}] = M/MBG). S_I was then calculated as MCR/log MSI during the OGTT. Thus, the S_I value, as defined here, is an approximate numerical index of the curve relating the MCR to the log of insulin during clamp tests in a test where a constant OGL is given. In our hands, S_I calculated from the OGTT correlated with S_I calculated from the frequently sampled intravenous glucose tolerance test (FSIVGTT) with minimal model analysis ($r = 0.72$, $P < 0.0001$, $n = 46$) (J.M.F.-R., W.R., R.C., unpublished observations).

Insulin resistance was also calculated through the fasting insulin resistance index (FIRI). FIRI = fasting glucose (mmol/l) × fasting insulin (mU/l)/25. In our experience, FIRI fairly correlates with the S_I calculated from FSIVGTT with minimal model analysis ($r = 0.79$, $P < 0.0001$, $n = 46$) (J.M.F.-R., W.R., R.C., unpublished observations).
Analytical methods. The serum glucose level during the OGTT was measured in duplicate by the glucose oxidase method with a glucose analyzer 2 (Beckman, Brea, CA). The coefficient of variation (CV) was 1.9%. The serum insulin level was measured in duplicate by monoclonal immunoradiometric assay (IRMA) (Medgenix Diagnostics, Fleunes, Belgium). The lowest limit of detection was 4.0 mU/l. The intra- and interassay CVs were 5.2 and 6.9%, respectively.

The Medgenix sTNF-R1 and sTNF-R2 solid-phase enzyme-amplified sensitivity immunoassays (EASIAs) (BioSource Europe, Fleunes, Belgium) were performed on microtiter plate. The minimum detectable concentration was estimated to be 0.1 ng/ml and was defined as the sTNFR1 or sTNFR2 concentration corresponding to the average optical density of 20 replicates of the zero standard + 2 SDs. The intra- and interassay CVs were <7 and <9%. sTNFR1 EASIA does not cross-react with sTNF-R2. TNF-α does not interfere with the assay.

Plasma TNF-α was measured using IRMA (Medgenix Diagnostics). Serum creatine kinase (CK) activity was measured by standard routine assays through the velocity of ATP synthesis when the enzyme is in contact with phosphocreatine and ADP.

Total serum cholesterol was measured through the reaction of cholesterol esterase/cholesterol oxidase/peroxidase (24). VLDL cholesterol was measured after ultracentrifugation at 45,000g. HDL cholesterol was quantified after precipitation with polyethylene glycol at room temperature (25). Total serum triglycerides were measured through the reaction of glycerol-phosphate-oxidase and peroxidase (26). VLDL triglycerides were measured after ultracentrifugation at 45,000g. LDL cholesterol was calculated as total cholesterol – (VLDL cholesterol + HDL cholesterol).
Statistical analyses. Descriptive results of continuous variables are expressed as means ± SD. Non-Gaussian-distributed variables were log$_{10}$ transformed to achieve normality. This applied to fasting levels of insulin, FIRI, S_I from the OGTT, sTNFR1, and sTNFR2. Relationships between variables were sought by Pearson's correlation coefficient and stepwise multivariate linear regression analysis with forward selection. The regression coefficient generated by this analysis indicates the slope of the association between the dependent variable and the specified independent variable, after adjusting for other independent variables in the model. The SE represents the variability in this association, and the significance is reflected by the P value. Comparison of variables across lean and obese men and women was performed by one-way analysis of variance using Fisher's test for multiple comparisons. Levels of statistical significance were set at $P < 0.05$.

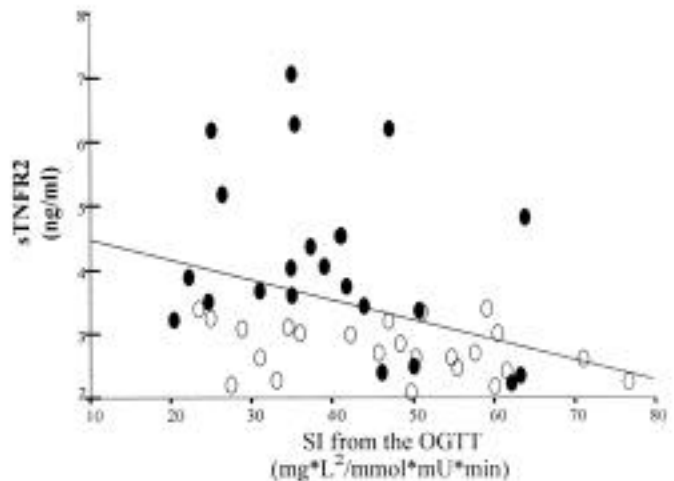All these analyses were performed with the BMDP statistical package (BMD Statistical Software, Cork, Ireland).

RESULTS

Anthropometric and biochemical characteristics of the subjects are shown in Table 1. Time since diagnosis of MyD had no effect on any of the parameters measured. In our study sample, no significant correlations between either sTNFR1 or sTNFR2 and BMI were found in either control subjects ($r = 0.08$ and $r = 0.29$ [NS], respectively) or MyD patients ($r = 0.24$ [NS] and $r = 0.36$ [$P = 0.09$], respectively). This is in contrast to previous observations in subjects with significantly higher BMI (17,18) or with a wider range of BMI than in this study sample (18). By excluding subjects with higher BMI and sTNFR2 levels, we are weakening the relationship between BMI and sTNFR2. In MyD patients, the lack of association was due to disproportionately increased sTNFR2 levels in patients with low BMI.

Both men and women MyD patients showed higher plasma insulin levels than control subjects in the presence of comparable glucose concentrations. sTNFR2, but not sTNFR1, levels were ~1.5-fold higher in MyD patients (Table 1). In parallel with these findings, FIRI was also higher in MyD patients. In fact, in the whole population, fasting insulin and FIRI strongly correlated with sTNFR2 in both men ($r = 0.77$ and $r = 0.81$, $P < 0.0001$, respectively) and women ($r = 0.67$ and $r = 0.64$, $P = 0.001$, respectively) ($r = 0.57$, $P < 0.0001$, in all subjects) (Fig. 1). sTNFR2 levels were also associated with the S_I calculated from the OGTT ($r = -0.43$, $P = 0.006$) (Fig. 2). We constructed a multiple linear regression model to predict FIRI, with BMI, WHR, and sTNFR2 as independent variables. In this model, both BMI ($P = 0.0014$) and sTNFR2 ($P = 0.0048$) levels contributed independently to 46% of the variance of FIRI. In another model, in which FIRI was substituted for S_I from the OGTT, both BMI ($P = 0.0001$) and sTNFR2 ($P = 0.04$) levels contributed independently to 48% of the variance of S_I from the OGTT.

Plasma TNF-α levels were below the linear range of the assay in 55% of the patients. This finding precludes a correct interpretation of circulating TNF-α.

Plasma LDL cholesterol and triglyceride concentrations were significantly increased in MyD patients (Table 1). However, it is important to note that LDL measurements were inferred by subtraction, and this approach may not be accurate given the elevated triglycerides. sTNFR1 and sTNFR2
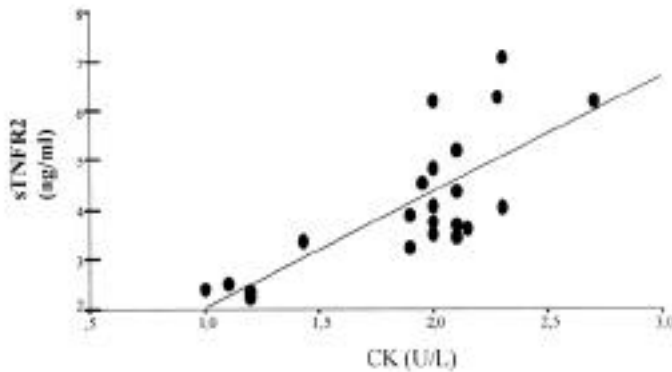
FIG. 3. Linear correlations between circulating sTNFR2 levels and serum CK in MyD patients ($r = 0.75$, $P < 0.0001$, $n = 22$).

levels were found to be strongly associated with plasma cholesterol, LDL cholesterol, and triglycerides (Table 2). Plasma sTNFR1 and sTNFR2 levels also correlated with serum CK activity in MyD patients ($r = 0.57$ [$P = 0.006$] and $r = 0.75$ [$P < 0.0001$] for sTNFR1 and sTNFR2, respectively) (Fig. 3).

DISCUSSION

The correlation between fasting insulin, FIRI, the S_I obtained from the OGTT, and sTNFR2 suggests that both glucose and sTNFR2 might be regulated by the same related signal. In fact, increased expression of TNF-α strongly correlates with the level of hyperinsulinemia (8) and the glucose disposal rate during euglycemic clamp technique (9). Because TNF-α is a strong inducer of TNFR2 expression in adipocytes (17) and other cell types (27), the negative correlation between insulin sensitivity and sTNFR2 might be attributed to increased TNF-α action in situations of insulin resistance. Marked hyperinsulinemia after OGTT is well known in MyD patients (3,4,28), and here we describe, for the first time to our knowledge, a relationship between insulin action and plasma sTNFR2 concentration in MyD patients.

sTNFRs are present constitutively in serum at concentrations that increase significantly in both inflammatory and noninflammatory disease states (29). TNFRs may in some situations inhibit the effects of TNF, in others, serve as carriers for TNF, and in some cases they may even augment the effects of TNF by prolonging its function (16). The study of the mechanisms that control TNFR function will provide new insights in the pathogenesis of insulin action in general and in the etiology of insulin resistance in MyD.

Glucose transport and oxidation are decreased in adipose tissue—the main metabolic target of TNF-α—in MyD patients (30). A decreased phosphorylation of muscle sar-

colemma due to deficient PK activity in muscle obtained from MyD patients has been described (31). Although this deficient PK activity is probably of genetic origin (2), it will be important to study whether these alterations in PK are amplified by increased TNF-α action (comparable to the reduction in tyrosine kinase activity of the insulin receptor induced by TNF-α [10]). The association between sTNFRs and CK is in concordance with the action of recombinant human TNF-α in vivo, which yielded higher serum CK values after its intravenous administration in melanoma patients (32).

In the last years, it has been demonstrated that TNF-α has important effects on whole-body lipid metabolism (33). TNF actions are thought to cause hypertriglyceridemia due to decreased lipolysis and increased VLDL secretion (33). Hypertriglyceridemia is well described in patients with the acquired immunodeficiency syndrome (34) or cystic fibrosis (35), two states of inferred high TNF-α action. Here we describe increased concentrations of plasma triglycerides and cholesterol levels in parallel with sTNFR1 and sTNFR2 concentrations in MyD patients. Interestingly, proinsulin-insulin hypersecretion has already been found to correlate with the levels of cholesterol and triglyceride in MyD (36). We speculate that this latter association is dependent on, and secondary to, increased TNF-α action. TNF-α has been found to produce a 25% increase in serum cholesterol levels and a 2.3-fold increase in hepatic hydro-3-methyl-glutaryl CoA reductase activity in C57Bl/6 mice (37). TNF-α is also capable of inducing sterol regulatory element binding protein-1 maturation, a key transcription factor in cholesterol biosynthesis, in a time- and dose-dependent manner (38). In conditions in which acute TNF secretion is not induced, increased cholesterol levels might be the first event, with TNF hypersecretion being secondary to lipid derangement. In this sense, the accumulation of cholesteryl esters in macrophages exposed to LDL immune complexes is associated with increased synthesis and release of TNF-α (39). Increased TNF secretion has also been observed in hypercholesterolemic rabbits (40) and in LDL receptor knockout mice, in which plasma TNF-α levels are elevated in parallel with LDL cholesterol (41). Furthermore, atherogenic diets enhance endotoxin-stimulated TNF-α gene expression in rabbit aortae (42). The implications of our findings are potentially important in the sense that sTNFR1 and sTNFR2 could constitute markers of atherosclerosis.

In summary, plasma sTNFR1 and sTNFR2 levels are associated with several metabolic abnormalities in MyD patients. Whether TNF action is implicated in the pathogenesis of MyD or is a simple marker of disease activity awaits further studies.

TABLE 2
Correlation between sTNFR1, sTNFR2, and lipid parameters

	Cholesterol	LDL cholesterol	Triglycerides
sTNFR1	0.58 ($P < 0.001$)	0.62 ($P < 0.0001$)	0.41 ($P < 0.005$)
sTNFR2	0.60 ($P < 0.0001$)	0.65 ($P < 0.0001$)	0.59 ($P < 0.0001$)

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