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MINI REVIEW

# The detection and role of heat shock protein 70 in various nondisease conditions and disease conditions: a literature review

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**Abstract** As an intracellular polypeptide, heat shock protein 70 (HSP70) can be exposed on the plasma membrane and/or released into the circulation. However, the role of HSP70 in various nondisease and disease conditions remains unknown. Quantitative methods for the detection of HSP70 have been used in clinical studies, revealing that an increase in circulating HSP70 is associated with various types of exercise, elderly patients presenting with inflammation, mobile phones, inflammation, sepsis, chronic obstructive pulmonary disease, asthma, carotid intima-media thickness, glutamine-treated ill patients, mortality, diabetes mellitus, active chronic glomerulonephritis, and cancers. Circulating HSP70 decreases with age in humans and in obstructive sleep apnea, arteriosclerosis, atrial fibrillation (AF) following coronary artery bypass surgery, nonalcoholic fatty liver disease, moderate-to-severe alcoholic fatty liver disease, hepatic steatosis, and *Helicobacter pylori* infection. In conclusion, quantitative methods can be used to detect HSP70, particularly in determining circulating HSP70 levels, using more convenient and rapid screening methods. Studies have shown that changes in HSP70 are associated with various nondisease and disease conditions; thus, HSP70 might be a novel potential biomarker reflecting various nondisease conditions and also the severity of disease conditions. However, the reliability and accuracy, as well as the underlying mechanism, of this relationship remain poorly understood, and large-sample clinical research must be performed to verify the role.

**Keywords** Heat shock protein 70 · Inflammation · Biomarkers · Nondisease conditions · Disease conditions

Heat shock proteins (HSPs) are ubiquitously synthesized in virtually all species and are phylogenetically conserved molecules. Although their function has typically been regarded as exclusive for intracellular molecules, it is now apparent that HSPs can be released from cells in the absence of cellular necrosis, and it is hypothesized that they might have beneficial health effects. HSPs also appear to be involved in diverse biological activities, such as apoptosis, carcinogenesis, and protection against cytotoxic damage. The synthesis of HSPs is highly up-regulated under environmental, physiological, and pathophysiological stress; indeed, the exposure of cells to physical or chemical stress results in increased synthesis of HSPs, particularly the heat shock protein 70 (HSP70) family. The HSP70 family functions as a molecular chaperone and reduces the stress-induced denaturation and aggregation of intracellular proteins (Rokutan 2000). HSP70 is also a member of the molecular chaperone family, which is involved in protecting cells against various types of stress. In addition, HSP70 has a protective role in tissue injury: it is produced in the liver and spleen as an acute-phase reactant and is released into the circulation to facilitate the disposal of dying cells (Merchant and Korbelik 2011). HSPs have been reported to play important roles in the activation and maturation of DCs (Tsan and Gao 2009). Increased serum levels of HSP70 reflect systemic inflammation and oxidative stress, and increases in HSP70 might dampen T cell-mediated inflammatory reactions in many clinical conditions (Stocki et al. 2012). The HSP70-enhanced suppression of Tregs may prevent exaggerated

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immune responses and may play a major role in maintaining immune homeostasis (Wachstein et al. 2012). In addition, HSP70 has been suggested to exert its cytoprotective action by protecting mitochondria and by interfering with stress-induced apoptotic programming (Merchant and Korbelik 2011). Quantitative studies have confirmed numerous detection methods for HSP70, and HSP70 has been associated with various nondisease and disease conditions, demonstrating its clinical significance.

## Detection methods for HSP70

HSP70 can be exposed on the plasma membrane and/or released into the circulation (Gruden et al. 2013). Therefore, the major detection methods for HSP70 have included extracellular fluid-based enzyme-linked immunosorbent assays (ELISAs) (Jenei et al. 2013a; Rejdak et al. 2012; Chebotareva et al. 2014; Agnew 2011; Cui et al. 2015) for plasma and immunofluorescence (Cui et al. 2015), western blotting (Cui et al. 2015), qRT-PCR (Gómez-Chocoa 2014), and flow cytometry (Cui et al. 2015; Agnew 2011; Gómez-Chocoa 2014) for plasma or urine, as well as immunohistochemical analysis (Yang et al. 2010; Gehrmann et al. 2014a; Omar et al. 1990) of various tissues and cells. ELISA is an optimized method for detecting serum concentrations of HSP70. Hence, the detection of HSP70 can be used to reflect various nondisease and disease conditions.

## Changes in the significance of HSP70 in various nondisease and disease conditions

### Changes in the significance of HSP70 levels in nondisease conditions

Exercise increases HSP family proteins, anti-oxidants, and anti-apoptotic proteins, and, running on a horizontal plane surface, can up-regulate the concentration of stress biomarker HSPs. HSP70 might participate in physiologic responses, such as fatigue induced by some types of physical exercise and heat acclimation. The level of plasma HSP70 during exercise is increased, and the enormous release of HSP70 from lymphocytes during high-load exercise might contribute to fatigue sensations (Heck et al. 2011). Heat exercise results in an increase in plasma HSP concentrations (Sandström et al. 2008; Horn et al. 2007). When 11 healthy male subjects underwent 11 days of heat acclimation, this state was achieved in the absence of significant changes in mean intracellular HSP70 fluorescence intensity or the percentage of HSP70+ lymphocytes during acclimation (Hom et al. 2012). Furthermore, 3 months of moderate physical exercise were sufficient to restore the normal expression of HSPs in adipose tissue, with a concomitant attenuation in the inflammatory response (Tiss et al. 2014). Uphill (predominantly concentric) exercise increases HSP70 beyond the eccentric type, and horizontal running was found to be a less-HSP70-responsive stimulus (Lollo et al. 2013). The mechanism by which exercise results in increases in HSP70 might be due to systemic alterations, such as hypoxia, increases in temperature, and the production of free radicals (Lollo et al. 2013). Exercise preconditioning has the beneficial effect of inhibiting oxidative stress and apoptotic cell death in the kidneys resulting from ischemic injury, even under conditions of HSP70 deficiency (Lee et al. 2013). A negative effect emerged with the increased use of mobile phones. A recent study demonstrated serum HSP and C-reactive protein (CRP) as systemic inflammatory biomarkers for mobile phone-induced radiation. Serum HSP70 levels and HSP70 gene expression were significantly higher in frequent users of mobile phones than in infrequent users; moreover, the serum HSP70 concentration was found to be independent of the duration of mobile phone exposure (Balakrishnan et al. 2014). Additionally, two- to four-fold increases in plasma levels of inflammatory mediators occur during the aging process, yet the association between serum HSP70 and age is unknown. A study in community-dwelling elderly people found that the serum concentration of HSP70 decreased with age in a normal population, with higher levels of HSP70 being associated with inflammation and frailty in elderly patients (Njemini et al. 2011a). Furthermore, healthy elderly individuals might have proinflammatory profiles that downregulate inducible HSP70 (Marotta et al. 2007). In addition, circulating serum HSP70 levels might serve as a marker for longevity (Terry et al. 2004). Hence, the detection of the circulating HSP70 level is helpful for observing inflammation in nondisease conditions.

### Changes in the significance of HSP70 levels in inflammation and sepsis

HSPs are highly conserved cytoprotective proteins. HSPs have been reported to be at elevated levels in the inflammation of RA patients (Schett et al. 2001; Van Herwijnen et al. 2013) and various infectious diseases caused by bacterial and protozoan pathogens (Dzaman-Serafin et al. 2005; Srivastava 2002). Additionally, HSP70 expression can be stimulated by febrile range temperature (FRT) (Gupta et al. 2013). There are positive correlations between the serum levels of HSP70 and various markers of inflammation. Hence, HSP70 is involved in inflammatory diseases, and the serum level of HSP70 is directly linked to the inflammatory status of the subject (Njemini et al. 2004). Serum HSP70 levels also undergo significant increases with increasing degrees of inflammation (Njemini et al. 2011b). Furthermore, increased serum HSP70 levels were associated with mortality in sepsis (Gelain et al. 2011). At the same time, exogenous HSP70 can be used for

the prophylactic treatment of various types of sepsis (Vinokurov et al. 2012). The mechanisms (Aschkenasy et al. 2011; Schroeder et al. 1999) involved suggest that HSP70 can impair apoptotic cellular pathways via interactions with caspases; HSP70 causes impairment of peripheral blood lymphocytes (including T and B lymphocytes), consequently resulting in immune dysfunction and severe secondary sepsis.

HSP70 has been implicated in the pathogenesis of chronic obstructive pulmonary disease (COPD) and asthma. A case-control study showed that higher levels of plasma HSP70 might be associated with an increased risk of COPD among coal workers and that this protein could potentially serve as a monitoring marker for COPD in coal workers (Cui et al. 2015). Another case-control study in patients with persistent bronchial asthma (Hou et al. 2011) indicated that induced sputum and plasma HSP70 could serve as useful markers for assessing the degree of airway obstruction in patients with asthma. Additionally, another case-control study in patients with asthmatic pregnancy demonstrated an elevation in circulating HSP70 levels compared to healthy pregnant women (Tamási et al. 2010). Obstructive sleep apnea (OSA) is associated with various diseases due to such symptoms as intermittent hypoxemia, oxidative stress, and sleep fragmentation. Patients with OSA might have lower levels of HSP70, consequently resulting in maladaptive responses to acute stress (Lavie et al. 2010). However, further studies are needed to establish the role of circulating HSP70 in the pathogeneses of COPD, asthma, and OSA.

## Changes in the significance of HSP70 levels in arteriosclerosis and AF following coronary artery bypass surgery

Atherosclerosis, an inflammatory-related disease, is a major cause of cardiovascular disease and of morbidity and mortality in humans. Previous reports have shown that the circulating levels of HSP are elevated in peripheral artery disease and cardiovascular diseases, and various studies have shown a correlation between extracellular HSP70 concentrations and the development of atherosclerosis. Furthermore, HSP70 might play a dual role in atherosclerosis. A randomized study (Dulin et al. 2010) demonstrated that extracellular HSP70 and anti-HSP60 were significantly lower in a population with atherosclerosis and that both proteins might serve as biomarkers for the progression of atherosclerotic disease. However, other studies have drawn the opposite conclusion: i.e., increases in carotid intima-media thickness at follow-up were less prevalent in subjects with high serum HSP70 levels at the time of enrollment (Pockley et al. 2003), and serum HSP70 levels were correlated with the severity of atherosclerosis in patients with carotid artery disease and chronic lower limb ischemia (Krepuska et al. 2011). These data support a putative role for plasma HSP70 in the development of arterial calcification, with HSP70 protecting against or modifying the progression of atherosclerosis. To date, the mechanisms of HSP70 in arteriosclerosis and atherosclerotic calcification remain unknown. On the one hand, the mechanism involves HSP70 acting as a cytoprotector and chaperone and playing a role as an anti-apoptotic protein, reducing the levels of Bax and AIF proteins synthesized in the cells of hypoxic tissue (Goel et al. 2010). On the other hand, there is a strict association between HSP70 and HDL levels (Assmann and Gotto 2004). There are elevated titers of anti-HSP antibodies in dyslipidemia and obesity (Ghayour-Mobarhan et al. 2007), and increased circulating levels of HSP70 might play a role in the initiation and/or progression of atherosclerosis in CKD subjects via CD4(+)CD28(null) cell perturbation (Yadav et al. 2013). Previous studies have implicated the dysregulation of transforming growth factor beta (TGF-β) signaling in the pathogenesis of a variety of diseases. Accordingly, extracellular HSP70 can interact with smooth muscle cells, inducing TGF-β1 synthesis and subsequent changes in the vascular extracellular matrix (González-Ramos et al. 2013). By impeding Smad2 phosphorylation, HSP70 has an essential role in TGF-β-induced epithelial-mesenchymal transition (EMT) (Li et al. 2011). In addition, the geldanamycin-mediated induction of HSP70 and its subsequent interaction with TGF-β receptors play a crucial role in the inhibition of TGF-β signaling (Yun et al. 2010). These data might reveal the mechanisms by which HSP70 contributes to the inflammation and fibrosis observed in atherosclerosis and other fibrosis-related diseases. However, its exact mechanisms require further investigation.

Atrial fibrillation (AF) after cardiac surgery is a common arrhythmia and is associated with a two-fold increase in cardiovascular mortality and morbidity. A prospective study found out that preoperative and postoperative circulating HSP70 levels were associated with postoperative AF (Oc et al. 2008). Moreover, AF recurrence rates were significantly higher after AF catheter ablation, a time when HSP70 levels increased to ≥0.025 ng/ml (Kornej et al. 2013). Hence, circulating HSP70 was found to be associated with AF following coronary artery bypass surgery (CABG), AF progression, and AF recurrence following catheter ablation. A previous study showed the immediate release of HSP70 into the circulation and the modulation of monocyte Toll-like receptor (TLR)-2 and TLR-4 expression after coronary artery bypass grafting (CABG). The authors speculated that TLR-4 and CD14 might be involved in an HSP70-mediated activation of innate immunity (Brit et al. 2002) and that HSP70 binds to human aorta smooth muscle cell TLR-4, which up-regulates the activator protein-1 (AP-1)-dependent transcriptional activity of the TGF-β1 promoter (Zhao et al. 2012). Consequently, HSP70 might be involved in the inflammation and fibrosis present in atherosclerosis and atherosclerosis-related diseases. Additionally, HSP70 plays a critical role in protecting the heart. Ischemia/reperfusion (I/R) can suppress HSP70 and

increase HSP70-interacting protein (CHIP) in cardiomyocytes, indicating that CHIP may be a potential target in the prevention of I/R-induced heart cell injury. Regardless, predicting the survival of a patient with heart failure (HF) and the prognoses of comatose postcardiac arrest patients are complex problems in clinical practice. In Kaplan-Meier survival analyses, HSP70 levels greater than the median were associated with significantly increased mortality, and multivariate survival models have shown that HSP70 levels greater than the median are an independent predictor of 5-year mortality in HF (Jenei et al. 2013a). However, extracellular HSP70 levels are significantly decreased in survivors, whereas they persist in nonsurvivors, predicting 30-day mortality regardless of age, sex, complications, or APAC HE II score (Jenei et al. 2013b). Thus, HSP70 might be a useful marker for estimating survival in patients with HF and in determining the prognosis of comatose postcardiac arrest patients.

### Changes in the significance of HSP70 levels in obesity, nonalcoholic fatty liver disease, alcoholic fatty liver disease, and hepatic steatosis

Nonalcoholic fatty liver disease (NAFLD) and hepatic steatosis (HS) increase the risks of both type 2 diabetes and cardio-cerebrovascular disease. Furthermore, obesity, NAFLD, alcoholic fatty liver disease, and HS are associated with inflammation. Obesity triggers the differential regulation of various heat shock responses in nondiabetic subjects (Tiss et al. 2014), and decreased heat shock transcription factor-1 (HSF1) levels in the liver and adipose tissue of obese patients correlate with the impairment of HSP70 in NAFLD. This impairment may affect HSP70-dependent anti-inflammation, with consequent oxidative stress and insulin resistance in advanced stages of NAFLD (Di Naso et al. 2015). Additionally, the median serum concentration of HSP70 in obese patients with NAFLD was found to be notably lower than that in controls. In a subgroup analysis, there was a significant distinction in the HSP70 levels of patients with the mildest grade of HF and more severe cases (Tarantino et al. 2012). Accordingly, HSP70 has been associated with NAFLD and HS, and HSPs might participate in the pathogenesis of alcoholic liver disease. The author of one study found that the median serum level of HSP70 in moderate-to-severe alcoholic fatty liver disease (AFLD) patients was significantly lower than in patients with mild AFLD or alcohol consumption without AFLD (Bao-Ge et al. 2015). Furthermore, immunostaining of histologic sections of hepatocytes revealed that many of these cells have increased positive immunoreactivity for HSP70; hence, immunocytochemical detection of HSP70 could serve as a more sensitive indicator of hepatocellular injury in AFLD (Omar et al. 1990). Kupffer cells are a site of HSP70 inhibition in NAFLD patients (Di Naso et al. 2015). Patients with stable alcoholic chronic liver disease show an attenuation of TLR2-mediated innate immune responses in peripheral blood monocytes, which may represent an important mechanism for acquired immunodeficiency (Pimentel-Nunes et al. 2010). In addition, peripheral blood $CD4^{+}CD25hiCD127$-/lo regulatory T cells (Tregs) are significantly decreased in patients with alcoholic hepatitis when compared with both healthy individuals and chronic alcoholic patients without liver disease (Almeida et al. 2013). Furthermore, circulating monocytes from actively drinking patients show abnormally low spontaneous and stimulated productions of inflammatory cytokines (Laso et al. 2007). Taken together, an impairment of anti-inflammation and immunodeficiency exists in NAFLD and ALD patients.

Changes in the significance of HSP70 levels in *H. pylori* infection, *H. pylori*-associated chronic gastritis, ulcerative colitis, the intestinal epithelial cell structure and function under cellular stress and injury, and glutamine-treated patients

*Helicobacter pylori* infection has been demonstrated to cause chronic gastric inflammation, which is associated with the progression of gastric ulcers and duodenal ulcer disease, as well as mucosa-associated lymphoid tissue lymphoma and gastric adenocarcinoma. One study suggested that overexpression of HSP70 plays an important role in protecting gastric cells against NH(2)Cl-induced injury (Oyake et al. 2006). *H. pylori* infection significantly attenuates the expression of HSP70 (Yeo et al. 2004); the mechanism might involve the induction of HSP70 for cytoprotection against *H. pylori* infection by inhibiting the expression of iNOS (Yeo et al. 2004; Liu et al. 2011). Moreover, the action of HSP70 might postpone monocyte apoptosis by protecting cytoplasmic and nuclear proteins from the damaging effects of bacterial products (Pierzchalski et al. 2014). A recent study indicated that HSP70 and HSP90 had potential applications for the assessment of the activity and prognosis of UC. Additionally, HSP70 and HSP90 can predict the presence of dysplasia and differentiate it from reactive atypia (Abou El Azm et al. 2015). HSP70 also plays a role in maintaining intestinal epithelial cells (Petrof et al. 2004; Yuan et al. 2010), acting against hypoxia/reoxygenation-induced apoptosis by increasing Bcl-2 expression and, in turn, inhibiting the mitochondria-related apoptotic pathway, which involves the disruption of the mitochondrial membrane potential and the release of cytochrome c (Yuan et al. 2010). Furthermore, serum HSP70 levels might be used to evaluate the clinical outcomes of critically ill patients. Glutamine is used to treat critical illnesses, and a recent study indicated that glutamine supplementation in critically ill children contributed to maintaining high HSP70 levels for a long period of time (Jordan et al. 2015). Glutamine (Gln)-supplemented parenteral nutrition significantly increased serum HSP70 in critically ill patients, and the magnitude of HSP70 enhancement in Gln-treated patients was correlated with improved clinical outcomes in patients in a surgical intensive

care unit (SICU) requiring parenteral nutrition (Ziegler et al. 2005). The molecular mechanism of Gln-induced HSP70 expression (Gong and Jing 2011) occurs through the enhancement of *O*-linked beta-*N*-acetylglucosamine modifications and the subsequent increases in the levels of endonuclear HSF-1 expression and HSF-1 transcription activity.

### Changes in the significance of HSP70 levels in diabetes mellitus and chronic glomerulonephritis

Diabetes mellitus is a disease with a large impact on public health. Recent data have indicated that the downregulation of HSPs in diabetic wounds is associated with wound healing impairment in type 2 diabetes mellitus (T2DM) subjects (Singh et al. 2015). Furthermore, serum HSP70 levels are increased in T1DM (Garamvölgyi et al. 2015) and T2DM patients (Garamvölgyi et al. 2015; Nakhjavani et al. 2012). In women with gestational diabetes mellitus, serum HSP70 levels are increased, and there is a significantly positive correlation with HbA1c levels (Garamvölgyi et al. 2015). Serum HSP70 and asymmetric dimethylarginine (ADMA) levels are significantly correlated in patients with high CRP levels (Nakhjavani et al. 2012), and a positive correlation between leptin and HSP was also observed in chronic inflammation, such as that in T2DM (Nakhjavani et al. 2013). One study showed increased HSP70 levels in diabetic patients with albuminuria (Morteza et al. 2013). Therefore, it could be hypothesized that associations between chronic inflammation with diabetes mellitus and diabetes mellitus-associated albuminuria exist. An increase in HSP70, potentially by targeting hyperglycemia-related deficits in HSF1 induction and activation in the liver, is a potent and viable strategy to improve glucose tolerance (Kavanagh et al. 2011). Despite hyperglycemia, the pancreas maintains HSP70 levels, likely in an attempt to protect vulnerable beta cells from exocrine pancreatic damage and from the stress associated with insulin hypersecretion (Kavanagh et al. 2009). Additionally, there is a relationship between active chronic glomerulonephritis and HSP70. Urinary HSP70 excretion was significantly higher in patients with higher chronic glomerulonephritis (CGN) activity and transient creatinine than in those with inactive nephritis, active CGN and preserved renal function, and persistent proteinuria and chronic renal failure (Chebotareva et al. 2014). The mechanism of HSP70 in patients with active CGN must be further investigated.

### Changes in the significance of HSP70 levels in stroke and seizure-related pathologic events

HSP70 can act as a danger signal and activate immune responses. HSP70 can also prevent the occurrence of apoptosis in the brain (Müller et al. 2007). Stronger HSP70 immunoreactivity was associated with smaller infarctions and better functional outcomes (Gómez-Choco et al. 2014). On the one hand, the neuro-protective effect is likely achieved via anti-apoptotic mechanisms in association with the overexpression of HSP70 (Zhao et al. 2014). Indeed, following experimental status epilepticus via the suppression of IκB kinase (IKK) activity and deactivation of IκBα, HSP70 protects against apoptotic cell death induced by nuclear factor-κB (NF-κB) activation and the nitric oxide synthase (NOS) II-peroxynitrite signaling cascade in hippocampal CA3 and glial cells (Chang et al. 2014). On the other hand, extracellular HSP70 can promote neuronal death by mediating the production of cytotoxic levels of tumor necrosis factor alpha, predominantly due to the Tlr4/Myd88 signaling cascade (Dvoriantchikova et al. 2014). Moreover, HSP70 is of potential value as a sensitive and specific biomarker of seizure-related pathologic events (Rejdak et al. 2012). Nonetheless, further study is needed to clarify these mechanisms.

### Changes in the significance of HSP70 levels in various cancers

HSPs are overexpressed in a broad range of human cancers, and they have been implicated in tumor cell proliferation, differentiation, invasion, metastasis, death, and recognition by the immune system (Ciocca and Calderwood 2005). Furthermore, HSP70 plays a role in the development of various cancers, and it could act as a potential tumor biomarker. Serum HSP70 levels were found to be consecutively increased in consecutive patients with chronic hepatitis, liver cirrhosis, and liver carcinomas, revealing a potential prognostic value (Yang et al. 2010), and are also typically positive in intrahepatic cholangiocarcinoma (IH-ChCa) and metastatic tumors (Lagana et al. 2013). As a consequence, the expression of HSP70 might be useful as a new prognostic marker for cholangiocarcinoma (Boonjaraspinyo et al. 2012). Serum levels of HSP70 and mortality in patients were found to be independent variables with high prognostic value for TNM stage, and they might be used to identify colorectal cancer patients at high risk for poor survival (Rozenberg et al. 2013). In addition, HSP70 cell surface expression was studied in patients with leukemia (Sedlackova et al. 2011), and patients with higher levels of HSP70 had significantly shorter survival in acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL), suggesting that HSP70 is an indicator of poor prognosis in these two acute diseases (Yeh et al. 2010). Furthermore, HSP70 overexpression possibly contributes to the avoidance of cell death, and HSP70 could be a key molecule for overcoming resistance to HSP90 inhibitors. Indeed, the combination of these two chaperones with conventional anti-cancer drugs is a promising therapeutic option for patients with advanced bladder cancer (Ma et al. 2014). HSP70 is a potential biomarker for the detection of tumors and for monitoring the clinical outcome of radiotherapy in squamous cell

carcinoma of the head and neck (SCCHN) patients (Gehrmann et al. 2014b). Hence, these data support the potential use of HSP70 as a biomarker in the diagnosis and treatment of cancers.

In conclusion, quantitative methods can be used for the detection of HSP70, particularly of circulating HSP70, and are convenient and rapid. Studies have found that changes in HSP70 are associated with various nondisease and disease conditions, and HSP70 might serve as a novel potential biomarker reflecting various nondisease conditions and the severity of disease conditions. However, its reliability and accuracy, as well as underlying mechanisms, remain poorly understood. Large-sample clinical studies are necessary to verify previously reported results.

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