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The relationship between heat shock protein 72 expression in skeletal muscle and insulin sensitivity is dependent on adiposity

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Abstract

Decreased gene expression of heat shock protein 72 (HSP72) in skeletal muscle is associated with insulin resistance in humans. We aimed to determine whether HSP72 protein expression in insulin-sensitive tissues is related to criterion standard measures of adiposity and insulin resistance in a young healthy human population free of hyperglycemia. Healthy participants (N = 17; age, 30 ± 3 years) underwent measurement of body composition (dual-energy x-ray absorptiometry), a maximum aerobic capacity test (Vo_{2max}), an oral glucose tolerance test, and a hyperinsulinemic-euglycemic clamp (M) to access insulin sensitivity. Skeletal muscle and subcutaneous adipose tissue biopsies were obtained by percutaneous needle biopsy. HSP72 protein expression in skeletal muscle was inversely related to percentage body fat ($r = -0.54$, $P < .05$) and remained significant after adjustment for age and sex ($P < .05$). Insulin sensitivity was also related to HSP72 protein expression in skeletal muscle ($r = 0.52$, $P < .05$); however, this relationship disappeared after adjustment for percentage body fat ($P = .2$). In adipose tissue, HSP72 protein expression was not related to adiposity or insulin sensitivity. Physical activity and aerobic fitness did not show any association with HSP72 protein expression in either tissue studied. A lower expression of HSP72 protein in human skeletal muscle was associated with increased adiposity and decreased insulin sensitivity in healthy individuals. These findings are consistent with rodent data suggesting that HSP72 stimulates fat oxidation with consequent reduction in fat storage and adiposity.

1. Introduction

Heat shock protein 72 (HSP72) is a cytoprotective chaperone protein currently of great interest with regard to type 2 diabetes mellitus because skeletal muscle HSP72 gene [1-3] and protein expression is lower in patients with type 2 diabetes mellitus compared with healthy individuals [4]. Furthermore, an inverse correlation exists between skeletal muscle HSP72 messenger RNA (mRNA) levels and insulin sensitivity as measured by hyperinsulinemic-euglycemic clamp [2,3]. Recently, we reported that inducing HSP72 expression via heat treatment, transgenic overexpression, or pharmacologic intervention in mice protects against obesity-induced insulin resistance [4]. These data were supported by the findings of Gupte and colleagues [5] who demonstrated that heat treatment improves glucose tolerance and prevents skeletal muscle insulin resistance in high-fat–fed rats.

The precise mechanisms by which induction of HSP72 protects against insulin resistance remain unknown; however, there is evidence that HSPs may reduce inflammation via c-Jun N-terminal kinase (JNK) [4-7] and/or the nuclear factor–κB (NF-κB) [8]. In addition, HSP72 may act through alternative mechanisms that include facilitation of fatty acid oxidation with a consequent reduction in fat storage. Consistent with this, mice with specific overexpression of HSP72 in skeletal muscle have decreased fat pad size and increased levels of oxidative enzymes [4], whereas in vitro heating of L6 muscle cells, which activates HSP72, can increase fatty acid oxidation and mitochondrial oxygen consumption [5].

No study has investigated the relationship between muscle and adipose HSP72 protein expression and adiposity in healthy humans. We aimed to determine whether HSP72 protein expression in insulin-sensitive tissues (vastus lateralis

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skeletal muscle and subcutaneous adipose tissue) is related to criterion standard measures of adiposity (dual-energy x-ray absorptiometry) and insulin sensitivity (euglycemic-hyperinsulinemic clamp) in a healthy population, free from the confounding effects of hyperglycemia.

2. Research design and methods

2.1. Study population

Individuals in the study were participants of a previous study into the pathogenesis of type 2 diabetes mellitus [9]. All were aged between 18 and 50 years, nondiabetic according to oral glucose tolerance test (OGTT) (World Health Organization 1999 criteria), unmedicated, nonsmokers, and, except for 7 with a body mass index (BMI) greater than 25 kg/m^2, healthy on physical examination. The protocol was approved by Alfred Hospital Ethics Committee and complied with the Declaration of Helsinki 2004. All subjects provided written informed consent before participation.

2.2. Experimental procedures

All participants underwent a medical screening, which included history, physical examination, and basic laboratory testing including fasting plasma lipid levels, liver function tests, anthropometric assessment, blood pressure, OGTT, maximum aerobic capacity (maximal oxygen uptake [$V_{O_{2max}}$]) and hyperinsulinemic-euglycemic clamp. Before testing, participants were asked to abstain from strenuous exercise and caffeine for 3 days. All metabolic testing was performed after a 12-hour overnight fast. All women were studied at the same time in the follicular phase of the menstrual cycle.

2.3. Anthropometric measurements

Body composition was estimated by total body dual-energy x-ray absorptiometry (DPX-L; Lunar Radiation, Madison, WI). Body mass index was calculated as a ratio of weight (in kilograms) and height^2 (in square meters). Waist and hip circumferences were measured, and waist-to-hip ratio (WHR) was calculated as an index of body fat distribution.

2.4. Metabolic studies

Maximal oxygen uptake was determined during a continuous incremental upright cycling test to volitional exhaustion on an electronically braked cycle ergometer (Ergo-line 900 ergometer, Bitz, Germany). Expired air was analyzed for volume, O_2, and CO_2 using calibrated analyzers (Cosmed Quark; b2, Rome, Italy). Habitual physical activity was assessed by a questionnaire (www.ipaq.ki.se). Two-hour 75-g OGTT was performed after a 12-hour fast. Plasma glucose concentrations were determined enzymatically (ELM 105, Radiometer, Copenhagen, Denmark). Lipid profile was measured by Cholestec LDX analyzer (Cholestec, Hayward, CA). Blood pressure was measured in triplicate (DINAMAP vital signs monitor, CRITIKON, Tampa, FL) after 30-minute rest. Insulin action was assessed at physiologic insulin concentrations during the hyperinsulinemic-euglycemic clamp [10,11]. A primed continuous intravenous insulin infusion (9 mU/kg) was administered for 3 hours at a constant rate of 40 mU/m^2 body surface area per minute (M). A variable infusion rate of glucose was adjusted to maintain blood glucose at a constant 5 mmol/L. The rate of total insulin-stimulated glucose disposal (M) was calculated for the last 40 minutes of the insulin infusions [9].

2.5. Muscle and adipose tissue biopsies

Biopsies of subcutaneous adipose and vastus lateralis muscle were performed using standard aseptic technique under local anesthesia as previously described [9].

2.6. Peripheral blood mononuclear cell isolation

Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood using Ficoll-Paque Plus density centrifugation (Amersham, Uppsala, Sweden). The PBMC pellet was resuspended in fetal bovine serum with 10% dimethyl sulfoxide and stored at −80°C.

2.7. Western blot analysis

Skeletal muscle and adipose tissue HSP72 and phosphorylated JNK (pJNK)/total JNK (tJNK) and PBMC pJNK/tJNK were determined by Western blotting. Muscle and adipose samples were lysed, and protein concentration was determined. Lysates were solubilized; and 40 μg of protein was loaded and resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis on polyacrylamide gels, transferred to membranes, and blocked with 5% milk. Immunoblotting was performed using primary antibodies (anti-HSP72 from Stressgen Bioreagents, Ann Arbor, MI; pJNK [Thr^{183}/Tyr^{185}], tJNK, and anti–β-actin used as a loading control purchased from Cell Signaling Technologies, Beverly, MA). Anti-mouse and anti-rabbit secondary antibodies were from Amersham Biosciences (Buckinghamshire, United Kingdom). The immunoreactive proteins were detected with enhanced chemiluminescence. Western blot bands were quantified using Optimas v.6.5.1 (MediaCybernetics, Bethesda, MD). Traced band area was normalized for background density and expressed as the product of band density and area.

2.8. Measurement of blood and tissue analytes

Blood samples for inflammation markers were drawn before the start of the clamp using standard phlebotomy techniques and immediately centrifuged (1500*g*, 15 minutes, 4°C), and plasma was stored at −80°C. Lincoplex kits (Linco Research, St Charles, MO) were used to determine plasma concentrations of leptin (Endocrine Kit) and adiponectin (Cardiovascular Disease Panel 1 kit). Assays were undertaken according to manufacturer's instructions on a Luminex 100 Bioplex machine (Bio-Rad, Atherton, CA), using Luminex Pro software (version 1.7). Plasma high-sensitivity

C-reactive protein (hsCRP) was measured by immunoturbidimetric assay on the Abbott Architect System (Abbott Park, IL). Enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN) were used to measure transforming growth factor (TGF)–β1, interleukin (IL)-1β, IL-10, IL-18, and tumor necrosis factor (TNF)–α (B-Bridge International, Mountain View, CA).

2.8.1. NF-κB activity measurements

Nuclear extraction from PBMCs, adipose, and skeletal muscle tissue was performed. The TransAM NF-κB DNA-binding activity assay (Active Motif, Carlsbad, CA) was used to detect and quantify NF-κB transcription factor activation, specifically of the p65 subunit as previously described [9].

2.9. Statistical analysis

Statistical analyses were performed using SAS (Cary, USA) Jump Statistics Software. Nonnormally distributed data were logarithmically transformed before approximate normal distributions. The relationships between HSP72 level and anthropometric and metabolic variables were examined using Pearson correlation coefficients. Multiple linear regression models and partial correlations were used to examine the relationships after adjusting for covariates. Differences between men and women were assessed by unpaired *t* test. Results are mean ± SEM (unless indicated).

3. Results

The anthropometric and metabolic characteristics of the study population are summarized in Table 1. Women had higher body fat (P = .004) and high-density lipoprotein concentrations (P = .02) and lower $V_{O_{2max}}$ (P = .03) than men. Interestingly, HSP72 protein expression in the skeletal muscle was not related to HSP72 in subcutaneous adipose tissue (representative blot, Fig. 2A) (r = 0.26, P = .3). Furthermore, HSP72 protein expression in skeletal muscle was not different between sex (P = .2).

3.1. Indicators of obesity

HSP72 protein expression in skeletal muscle was negatively related to percentage body fat before (Fig. 1A, r = −0.54, P < .05) and after adjustment for age and sex (P < .05). HSP72 protein expression in the skeletal muscle was not related to weight, BMI, or WHR (all Ps > .1). HSP72 protein expression in subcutaneous adipose tissue was not related to any of the anthropometric variables (all Ps > .2).

In agreement with the finding of a relationship between HSP72 protein expression in the skeletal muscle and percentage body fat, HSP72 was inversely related to plasma leptin levels before (r = −0.57, P < .05) and after adjustment for age and sex (Fig. 1B, P = .01) but was not independent of percentage body fat (P = .2). Adipose tissue HSP72 protein expression was not related to plasma leptin (P > .2).

Table 1
Anthropometric and metabolic parameters of study population

	All (n = 17)	Female (n = 7)	Male (n = 10)
Age (y)	30 ± 3	33 ± 5	28 ± 3
Weight (kg)	77.5 ± 2.9	72.0 ± 4.7	81.4 ± 3.2
BMI (kg/m^2)	26.2 ± 1.0	25.7 ± 1.7	26.5 ± 1.2
Waist circumference (cm)	86 ± 3	84 ± 5	87 ± 3
WHR	0.85 ± 0.02	0.80 ± 0.03	0.88 ± 0.08
% Body fat	28.4 ± 2.9	37.6 ± 2.7	21.9 ± 3.3*
Total cholesterol (mmol/L)	4.33 ± 0.15	4.56 ± 0.30	4.17 ± 0.15
High-density lipoprotein (mmol/L)	1.33 ± 0.10	1.60 ± 0.13	1.13 ± 0.12*
Low-density lipoprotein (mmol/L)	2.47 ± 0.15	2.44 ± 0.22	2.48 ± 0.22
Triglycerides (mmol/L)	1.20 ± 0.18	1.11 ± 0.22	1.26 ± 0.28
Systolic blood pressure (mm Hg)	117 ± 3	112 ± 4	122 ± 2
Diastolic blood pressure (mm Hg)	68 ± 2	65 ± 3	70 ± 3
Fasting glucose (mmol/L)	4.52 ± 0.14	4.26 ± 0.20	4.70 ± 0.17
2-h glucose (mmol/L)	4.77 ± 0.35	4.91 ± 0.71	4.67 ± 0.37
$V_{O_{2max}}$ (mL/[kg min])	30.6 ± 2.3	24.7 ± 3.0	34.7 ± 2.7*
Physical activity questionnaire (MET)	2864 ± 2368	2791 ± 2097	2924 ± 2664
Insulin sensitivity M (mg/[kg min])	9.7 ± 1.2	10.3 ± 2.4	9.3 ± 1.2

* Difference between sexes, P < .05.

3.2. Indicators of glucose metabolism

HSP72 protein expression in skeletal muscle was related to insulin sensitivity as measured by hyperinsulinemic-euglycemic clamps before (0.52, P < .05) and after adjustment for age and sex (Fig. 1C, P = .02). The relationship disappeared after adjustment for percentage body fat (P = .2). HSP72 protein expression in adipose tissue was not related to insulin sensitivity (r = −0.06, P > .2). HSP72 protein expression from both skeletal muscle and adipose was not related to fasting or 2-hour glucose after OGTT (all Ps > .05).

3.3. Exercise variables

HSP72 protein expression in the muscle was related to $V_{O_{2max}}$ (r = 0.48, P < .05), but the relationship disappeared after adjustment for age and sex (P = .7). When $V_{O_{2max}}$ was normalized to lean body mass and correlated to HSP72 skeletal muscle protein expression, we observe that the $V_{O_{2max}}$ correlation changes too (r = 0.30, P = .24). This is indicative of the female participants in the cohort having a higher body fat percentage. HSP72 protein expression in the muscle was not related to habitual physical activity (P > .1). HSP72 protein expression in adipose tissue was not related to $V_{O_{2max}}$ or habitual physical activity (both Ps > .2).

3.4. Plasma inflammation markers

Skeletal muscle HSP72 protein expression was not related to plasma hsCRP, TGF-β1, IL-1β, IL-18, or TNF-α (P > .1).

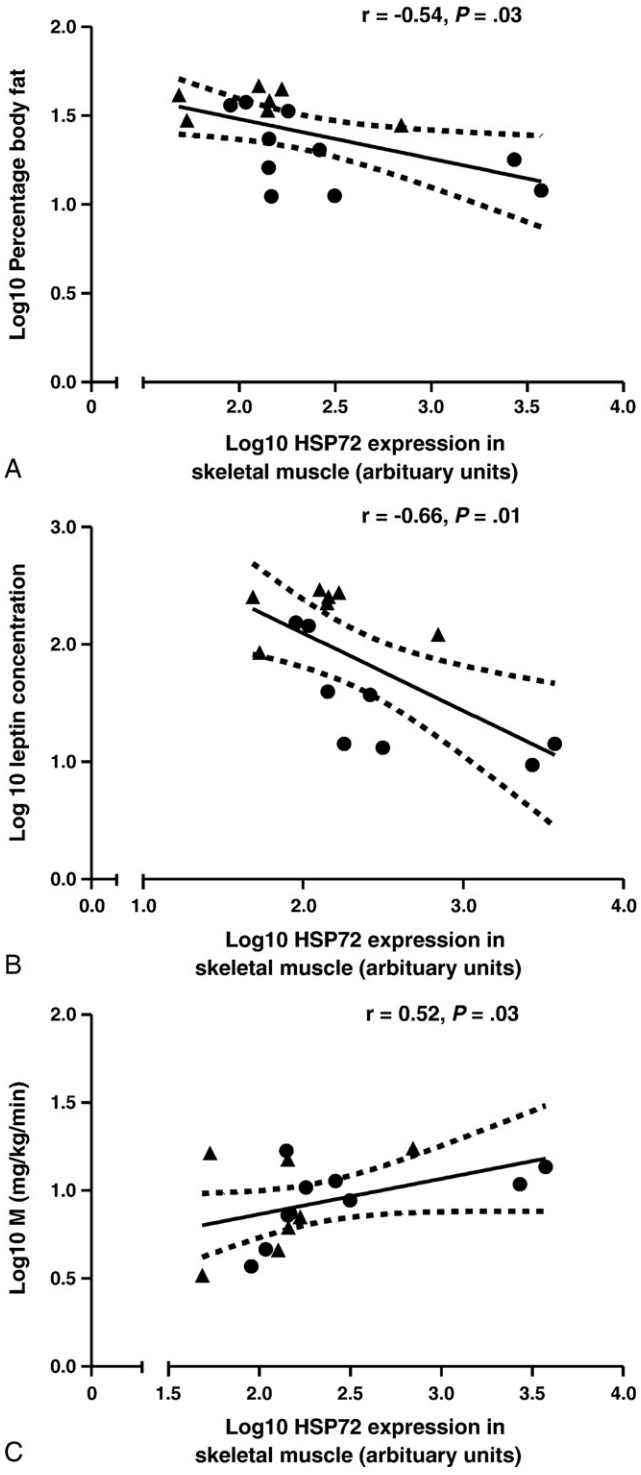

Fig. 1. Univariate correlations between HSP72 skeletal muscle expression and (A) percentage body fat (N = 17), (B) circulating leptin concentrations (n = 15), and (C) insulin sensitivity (N = 17). Triangles represent female participants, and circles represent male participants.

Adipose tissue HSP72 protein expression was also not related to any of these proinflammatory plasma markers ($P > .1$). Neither of the HSP72 protein expression in the skeletal muscle or adipose tissue was related to NF-κB activity or JNK phosphorylation in muscle, adipose tissue, or PBMCs (Table 2, all Ps > .1). A representative pJNK and tJNK blot is displayed in Fig. 2B. In terms of any relationship between HSP72 protein expression and markers of alternative activation (anti-inflammatory phenotype), HSP72 protein expression levels in both skeletal muscle and adipose tissue did not correlate significantly with either adiponectin or IL-10 levels (all Ps > .1).

Table 2
Univariate relationships between HSP72 skeletal muscle and adipose tissue expression and inflammatory markers

	HSP72 skeletal muscle	HSP72 adipose tissue
Circulation		
hsCRP	$r = 0.27$	$r = 0.29$
TGF-B1	$r = -0.08$	$r = 0.17$
IL-1B	$r = 0.20$	$r = 0.03$
IL-18	$r = -0.09$	$r = -0.03$
TNF-α	$r = 0.02$	$r = 0.08$
Adiponectin	$r = 0.28$	$r = 0.14$
IL-10	$r = -0.06$	$r = 0.17$
Skeletal muscle		
NF-κB	$r = 0.10$	$r = 0.14$
pJNK	$r = 0.39$	$r = 0.08$
Adipose tissue		
NF-κB	$r = 0.13$	$r = 0.00$
pJNK	$r = 0.10$	$r = 0.13$
PBMCs		
NF-κB	$r = -0.02$	$r = 0.17$
pJNK	$r = -0.19$	$r = -0.27$

4. Discussion

The current study demonstrates in humans that HSP72 protein expression in skeletal muscle is inversely correlated with percentage body fat and positively correlated with insulin sensitivity. Furthermore, we demonstrate that the association between HSP72 protein levels and insulin

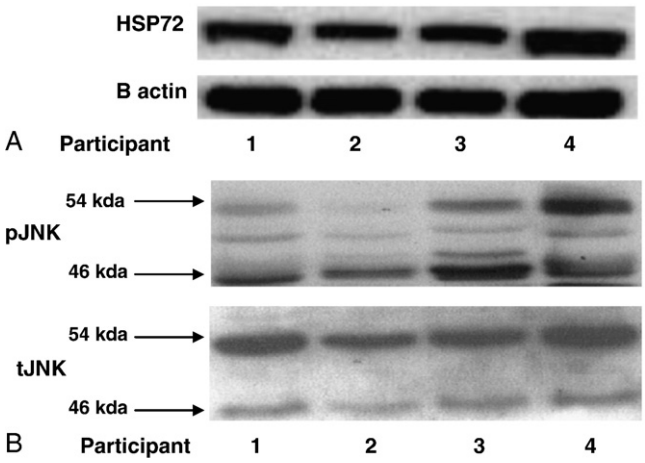

Fig. 2. Representative Western blots of human adipose tissue from 4 participants probed for (A) HSP72 and β-actin and (B) pJNK (Thr183/Thr185) and tJNK.

sensitivity is dependent on percentage body fat, suggesting that this relationship is mediated by adiposity. Moreover, we demonstrate that HSP72 protein expression was not related to markers of inflammatory pathways (NF-κB and JNK). Interestingly, these relationships were not observed in subcutaneous adipose tissue, indicating a specific skeletal muscle association.

HSP72 protein expression was related to percentage body fat but not weight, BMI, or WHR. In addition, our findings are further substantiated by a relationship between skeletal muscle HSP72 protein expression and leptin levels because leptin is known to be strongly correlated with body fat [12]. These data support animal and cell culture experiments suggesting that increased HSP72 expression in skeletal muscle promotes fatty acid oxidation and energy expenditure. Specifically, tissue-specific overexpression of skeletal muscle HSP72 decreases fat pad size and increases the levels of the oxidative enzymes citrate synthase and β-hydroxyacyl–coenzyme A dehydrogenase, reflecting increased fatty acid oxidation capacity in the muscles of these mice [4]. Thus, individuals with a predisposition, possibly genetic, for low HSP72 expression in skeletal muscle may have a decreased capacity for fatty acid utilization and increased adiposity that may promote development of insulin resistance.

The positive relationship between insulin sensitivity and HSP72 protein expression levels supports 2 previous studies that demonstrated that skeletal muscle HSP72 gene expression correlates with insulin-stimulated glucose disposal [2,3]. It was important to examine protein levels in the current study, as mRNA levels do not always correspond. Furthermore and importantly, the previous articles by Kurucz and colleagues [2] and Bruce and colleagues [3] did not adjust when assessing the relationship between HSP72 and insulin resistance for important confounding covariates including adiposity. In the current study, HSP72 in the skeletal muscle was related to insulin sensitivity before and after adjustment for age and sex, both factors known to influence insulin sensitivity. However, the relationship was not independent of percentage body fat, suggesting that it is mediated by adiposity. This suggests that this relationship between HSP72 protein expression and insulin sensitivity is dependent on the degree of adiposity.

Although in vitro and in vivo studies provide evidence that HSPs may act on inflammation by down-regulating the JNK [4-7] and NF-κB pathways [8], we observed no relationship between HSP72 protein expression and JNK phosphorylation or NF-κB activity in either muscle or adipose tissue. This is possibly due to these pathways not acting on each other's regulation in vivo in humans or the fact that participants being young and healthy had minimal inflammation and that there was no necessity for an adaptive heat shock protein response. The results may have been different if this study was performed in patients where inflammation is likely to be present such as those with type 2 diabetes mellitus and/or obesity. As such, the finding that inflammation does not correlate with HSP72 expression may be limited to normal healthy populations.

As HSP72 from the skeletal muscle and that from adipose tissue were unrelated to each other and they did not correlate with the same factors, we hypothesize that HSP72 expression levels in adipose are not as important as HSP72 expression in muscle in mediating protection against a diet high in fat and/or a sedentary lifestyle. This is consistent with skeletal muscle accounting for 70% to 90% of the glucose uptake after a glucose challenge [13,14], with adipose tissue responsible for less than 5% [15,16].

Limitations of this study include the small number of participants and the cross-sectional study design. HSP72 expression levels in visceral fat may also be of more relevance than the subcutaneous fat examined in this investigation. Several studies have indeed shown that it is the visceral fat rather than subcutaneous that is more inflamed and has deleterious effects on insulin sensitivity. Omentectomy (removal of visceral fat) results in decreased insulin and glucose levels in humans [17], whereas removal of subcutaneous fat by liposuction does not result in improvement in metabolic measures [18]. Although it is much more difficult to obtain visceral adipose samples than subcutaneous tissue, an investigation of HSP72 levels in visceral fat may reveal a different result.

In the present study, we have not provided direct support for our conclusions that HSP72 expression is strongly associated with skeletal muscle mitochondrial function and/or fatty acid oxidation capacity. Measurements of the activity of citrate synthase and β-hydroxyacyl–coenzyme A dehydrogenase, key enzymes of the tricarboxylic acid cycle and β-oxidation pathways, respectively, would be one such way to do so. In the article by Bruce and colleagues [3], the authors did perform the measurements of these enzymes; and they did correlate positively with the HSP72 mRNA levels. As we have now shown the protein levels of HSP72 to also correlate with insulin sensitivity, there is a high likelihood that they would also correlate with these enzymes in this population. Further research confirming our data in humans with regard to these enzymes are warranted.

In conclusion, we show that HSP72 protein expression in skeletal muscle was related inversely to adiposity and positively to insulin sensitivity. The relationship between HSP72 protein expression and insulin sensitivity is explained by adiposity. Further studies are warranted to determine potential mechanisms linking HSP72 in skeletal muscle, fat utilization, and consequent fat storage in adipose tissue.

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References

[1] Patti ME, Butte AJ, Crunkhorn S, Cusi K, Berria R, Kashyap S, et al. Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: potential role of PGC1 and NRF1. Proc Natl Acad Sci U S A 2003;100:8466-71.

[2] Kurucz I, Morva A, Vaag A, Eriksson KF, Huang X, Groop L, et al. Decreased expression of heat shock protein 72 in skeletal muscle of patients with type 2 diabetes correlates with insulin resistance. Diabetes 2002;51:1102-9.

[3] Bruce CR, Carey AL, Hawley JA, Febbraio MA. Intramuscular heat shock protein 72 and heme oxygenase–1 mRNA are reduced in patients with type 2 diabetes: evidence that insulin resistance is associated with a disturbed antioxidant defense mechanism. Diabetes 2003;52:2338-45.

[4] Chung J, Nguyen AK, Henstridge DC, Holmes AG, Chan MH, Mesa JL, et al. HSP72 protects against obesity-induced insulin resistance. Proc Natl Acad Sci U S A 2008;105:1739-44.

[5] Gupte AA, Bomhoff GL, Swerdlow RH, Geiger PC. Heat treatment improves glucose tolerance and prevents skeletal muscle insulin resistance in rats fed a high-fat diet. Diabetes 2009;58:567-78.

[6] Gabai VL, Meriin AB, Mosser DD, Caron AW, Rits S, Shifrin VI, et al. Hsp70 prevents activation of stress kinases. A novel pathway of cellular thermotolerance. J Biol Chem 1997;272: 18033-7.

[7] Park HS, Lee JS, Huh SH, Seo JS, Choi EJ. Hsp72 functions as a natural inhibitory protein of c-Jun N-terminal kinase. Embo J 2001;20: 446-56.

[8] Meldrum KK, Burnett AL, Meng X, Misseri R, Shaw MB, Gearhart JP, et al. Liposomal delivery of heat shock protein 72 into renal tubular cells blocks nuclear factor–kappaB activation, tumor necrosis factor–alpha production, and subsequent ischemia-induced apoptosis. Circ Res 2003;92:293-9.

[9] Sourris KC, Lyons JG, de Courten MP, Dougherty SL, Henstridge DC, Cooper ME, et al. JNK activity in subcutaneous adipose tissue but not NF{kappa}B activity in peripheral blood mononuclear cells is an independent determinant of insulin resistance in healthy individuals. Diabetes 2009;58:1259-65.

[10] DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol 1979;237:E214-23.

[11] DeFronzo RA, Tobin JD, Rowe JW, Andres R. Glucose intolerance in uremia. Quantification of pancreatic beta cell sensitivity to glucose and tissue sensitivity to insulin. J Clin Invest 1978;62:425-35.

[12] Larsson H, Elmstahl S, Ahren B. Plasma leptin levels correlate to islet function independently of body fat in postmenopausal women. Diabetes 1996;45:1580-4.

[13] DeFronzo RA, Jacot E, Jequier E, Maeder E, Wahren J, Felber JP. The effect of insulin on the disposal of intravenous glucose. Results from indirect calorimetry and hepatic and femoral venous catheterization. Diabetes 1981;30:1000-7.

[14] Baron AD, Brechtel G, Wallace P, Edelman SV. Rates and tissue sites of non–insulin- and insulin-mediated glucose uptake in humans. Am J Physiol 1988;255(6 Pt 1):E769-74.

[15] Bjorntorp P, Berchtold P, Holm J, Larsson B. The glucose uptake of human adipose tissue in obesity. Eur J Clin Invest 1971;1:480-5.

[16] Frayn KN, Coppack SW, Humphreys SM, Whyte PL. Metabolic characteristics of human adipose tissue in vivo. Clin Sci (Lond) 1989; 76:509-16.

[17] Thorne A, Lonnqvist F, Apelman J, Hellers G, Arner P. A pilot study of long-term effects of a novel obesity treatment: omentectomy in connection with adjustable gastric banding. Int J Obes Relat Metab Disord 2002;26:193-9.

[18] Klein S, Fontana L, Young VL, Coggan AR, Kilo C, Patterson BW, et al. Absence of an effect of liposuction on insulin action and risk factors for coronary heart disease. N Engl J Med 2004;350:2549-57.