Heat shock proteins: in vivo heat treatments reveal adipose tissue depot-specific effects

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Heat shock proteins: in vivo heat treatments reveal adipose tissue depot-specific effects. J Appl Physiol 118: 98–106, 2015. First published November 13, 2014; doi:10.1152/japplphysiol.00286.2014.—Heat treatments (HT) and the induction of heat shock proteins (HSPs) improve whole body and skeletal muscle insulin sensitivity while decreasing white adipose tissue (WAT) mass. However, HSPs in WAT have been understudied. The purpose of the present study was to examine patterns of HSP expression in WAT depots, and to examine the effects of a single in vivo HT on WAT metabolism. Male Wistar rats received HT (41°C, 20 min) or sham treatment (37°C), and 24 h later subcutaneous, epididymal, and retroperitoneal WAT depots (SCAT, eWAT, and rpWAT, respectively) were removed for ex vivo experiments and Western blotting. SCAT, eWAT, and rpWAT from a subset of rats were also cultured separately and received a single in vitro HT or sham treatment. HSP72 and HSP25 expression was greatest in more metabolically active WAT depots (i.e., eWAT and rpWAT) compared with the SCAT. Following HT, HSP72 increased in all depots with the greatest induction occurring in the SCAT. In addition, HSP25 increased in the rpWAT and eWAT, while HSP60 increased in the rpWAT only in vivo. Free fatty acid (FFA) release from WAT explants was increased following HT in the rpWAT only, and fatty acid reesterification was decreased in the rpWAT but increased in the SCAT following HT. HT increased insulin responsiveness in eWAT, but not in SCAT or rpWAT. Differences in HSP expression and induction patterns following HT further support the growing body of literature differentiating distinct WAT depots in health and disease. HSP72: metabolism; stress response; lipolysis; fatty acid reesterification

We previously demonstrated that in vivo heat treatments improve whole body glucose homeostasis and insulin-stimulated glucose uptake in skeletal muscle (12, 13). Heat treatments increased heat shock protein 72 (HSP72) expression in slow- and fast-twitch skeletal muscle in parallel with improvements in insulin-stimulated glucose uptake. HSP72 expression was also observed in epididymal adipose tissue and liver (12). Our findings and others demonstrate that in vivo heat treatments and transgenic overexpression of skeletal muscle HSP72 reduced epididymal fat pad mass in rodents fed a high-fat diet (3, 12), and heat treatment reduced plasma triglycerides and free fatty acids in db/db mice (17). Drew et al. (6) recently expanded upon these observations by showing that global knock-out of HSP72 induced an insulin-resistant phenotype with increased adipose tissue mass. Despite the observed induction of HSPs in epididymal adipose tissue, and the reduction in free fatty acids and adipose tissue mass with in vivo heat treatments, very little is known about the expression patterns and function of HSPs in adipose tissue.

HSPs are molecular chaperones that aid in protein refolding and prevent protein aggregation (9, 23). HSP72 is a cytosolic chaperone, is highly inducible following heat stress, and has anti-inflammatory and antiapoptotic properties (9, 23). HSP25 is another cytosolic chaperone that plays a role in cytoskeletal dynamics and also has anti-inflammatory functions (9, 23). HSP60 is largely a mitochondrial chaperone shown to be essential for proper folding of imported mitochondrial proteins (4, 23). HSP expression in skeletal muscle corresponds with oxidative capacity with higher constitutive, or basal/unstressed levels, of HSP expression observed in slow-twitch oxidative skeletal muscle compared with fast-twitch glycolytic skeletal muscle (2, 20, 21, 24). As a result, muscle fiber type and constitutive HSP expression levels play an important role in determining HSP function in skeletal muscle (12).

There are significant metabolic differences between white adipose tissue (WAT) present in the subcutaneous depots and WAT present in the abdominal cavity (retroperitoneal and epididymal depots) (10, 15). Adipocytes from abdominal depots have greater secretion of inflammatory cytokines (7, 15, 27, 38), greater lipolysis and fatty acid reesterification (1, 14, 19, 32), and greater oxygen consumption and mitochondrial protein content (5, 18). It is currently unknown as to whether HSP expression corresponds with metabolic activity in adipose tissue depots.

The purpose of the present study was twofold: first, to determine if HSP expression varied across WAT depots (subcutaneous, epididymal, and retroperitoneal); and second, to determine if the induction of HSPs following a single heat treatment was WAT depot-specific. We also examined the impact of an acute bout of heat treatment on adipose tissue lipolysis and insulin responsiveness. We hypothesized that HSP expression patterns would parallel the metabolic activity of the WAT depots, with greater HSP expression demonstrated in more metabolically active depots (i.e., retroperitoneal and epididymal depots) (10, 15). Additionally, we hypothesized that HSP induction would be greatest in WAT depots where constitutive HSP levels were low. These differences between WAT depots could have potential implications for HSP function in adipose tissue health and disease.

MATERIALS AND METHODS

Materials. HSP72 primary antibody (cat. no. SPA-810) was purchased from Enzo Life Sciences (Farmingham, NY). Phospho-HSP25 (Ser-82) (cat. no. 905-642), total HSP25 (cat. no. SPA-801), and HSP60 (cat. no. SPA-807) primary antibodies were purchased from Stressgen (Victoria, British Columbia, Canada). Phospho-AMPK

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Fresh fat depots from in vivo sham- and heat-treated animals and were normalized to microgram protein content measured by Bradford assay performed in triplicate to compare WAT depots. Epi stimulation increased glycerol and FFA release in all adipose tissue depots examined and was used as a positive control; however, Epi responsiveness was not changed following a single in vivo heat treatment (data not shown). Fatty acid reesterification was assessed by quantifying the ratio of FFA to free glycerol (FFA/glycerol) where a ratio near 3.0 represents an absence of fatty acid reesterification, and a ratio near 0.0 represents complete reesterification of fatty acids into triglycerides (19, 40). Absolute rates of fatty acid reesterification were calculated as the differences between 3 times glycerol (theoretical fatty acid release) and the measured fatty acid release (37, 39). This calculation assumes negligible fatty acid oxidation.

Adipose tissue organ culture. In a separate set of experiments, SCAT, eWAT, and rpWAT were removed and cultured as previously described (8, 31, 39, 40) from 14-wk-old male Wistar rats weighing 350–375 g each (n = 6). Under sterile conditions, adipose tissue from each depot was minced and placed into separate culture dishes (~500 mg of adipose tissue per dish), one dish designated for heat treatment per depot and one dish designated for sham treatment per depot. This was repeated for each of the six animals: sham treatment (n = 6) and heat treatment (n = 6) for each adipose tissue depot. Culture dishes contained 15 ml of M-199 supplemented with 1% antibiotic-penicillin-streptomycin, 50 μU/ml insulin, and 2.5 mmol/l dexamethasone. Culture dishes were placed in an incubator overnight maintained at 37°C with a gas phase of 5% CO₂. After a 24-h recovery incubation, adipose tissue cultures from each depot were placed either in a 42°C water bath for 30 min or maintained at 37°C. After heat treatment, cultures were placed back into the incubator and 24 h later culture medium containing the adipose tissue was poured into ice-cold PBS, filtered, and the remaining adipose tissue samples were frozen at −80°C.

Western blotting. WAT from in vivo sham- and heat-treated animals not used for ex vivo adipose tissue explants, as well as minces from adipose tissue organic cultures (ATOC) were frozen in liquid nitrogen and stored at −80°C to be processed for Western blotting by methods previously described (12, 13). WAT was homogenized in a 2:1 (volume-to-weight) ratio of ice-cold cell extraction buffer containing 10 mmol/l Tris-HCl (pH 7.4); 100 mmol/l NaCl; 1 mmol/l of each of EDTA, EGTA, NaF, and phenylmethylsulfonyl fluoride; 2 mmol/l NaVO₃; 20 mmol/l Na₃P₂O₇; 1% Triton X-100; 10% glycerol; 0.1% SDS; 0.5% deoxycholate; and 250 μl/5 ml protease inhibitor cocktail. Homogenates were sonicated for 3–5 s, rotated for 30 min at 4°C, and then centrifuged for 20 min at 3,000 rpm at 4°C. The infranatant was removed and protein concentration determined by Bradford assay performed in triplicate. Samples were diluted in HES buffer (20 mmol/l HEPES, 1 mmol/l EDTA, 200 mol/l sucrose, pH 7.4) and 5 × Laemmli buffer containing 100 mmol/l dithiothreitol.
(Thermo Scientific) based on protein concentration to generate samples containing the same concentration of protein for analysis by SDS-PAGE. Samples were heated in a boiling water bath for 5 min. Protein (10–30 μg) was separated on 7.5–10% SDS-PAGE gels, followed by a wet transfer to a nitrocellulose membrane for 60–90 min at 200 mA. Membranes were Ponceau stained to verify even protein loading. Primary antibodies were incubated for 1 h at room temperature in Tris-buffered saline, 0.1% Tween 20 (TBST), and 5% nonfat dry milk or 1% BSA, followed by an overnight incubation with the appropriate primary antibodies. Primary antibodies were diluted in TBST with 1% nonfat dry milk or 1–5% BSA at a concentration of 1:500 or 1:1,000. Following three brief washes with TBST, blots were incubated for 1 h at room temperature in TBST 1% nonfat dry milk supplemented with an appropriate HRP-conjugated secondary antibody at a concentration of 1:5,000 or 1:10,000. Blots were then washed twice with TBST and once with TBS. Blots were visualized by enhanced chemiluminescence (ECL). Bands were quantified using ImageJ densitometry. Blots for in vivo experiments were then stripped and re-probed for β-actin as a loading control. Blots for in vitro experiments were normalized to Ponceau staining. Blots were stripped for 15–20 min at 55°C in buffer containing 62.5 mmol/l Tris·HCl, 2% SDS, and 100 mmol/l 2-mercaptoethanol.

**Statistical analyses.** Results are presented as means ± SE. Statistical significance was set at $P < 0.05$ and analyses performed using Sigma Plot for Windows, version 12.0 (Systat Software, Chicago, IL). One-way ANOVAs were performed to test for significant differences

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**Fig. 1.** HSP expression and induction following a single heat treatment in vivo. Heat shock protein 72 (HSP72) (A), HSP25 (B), phospho-HSP25 (C), and HSP60 (D) levels between white adipose tissue (WAT) from subcutaneous, epididymal, and retroperitoneal depots (SCAT, eWAT, and rpWAT, respectively). Male Wistar rats received a single heat treatment (41°C for 20 min) or sham treatment (37°C for 20 min). Twenty-four hours following heat or sham treatment, WAT depots were removed and protein expression measured by Western blot. Protein levels were normalized to β-actin protein levels. *$P < 0.05$, **$P < 0.01$ denotes a significant increase following heat treatment determined by ANOVA. †$P < 0.05$, ††$P < 0.01$, †††$P < 0.001$ denotes that eWAT or rpWAT are significantly different from SCAT determined by ANOVA. #$P < 0.05$, ##$P < 0.01$ denotes that eWAT is significantly different from rpWAT determined by ANOVA. Values are means ± SE. $N = 3–6$ samples per group in SCAT; $N = 5–6$ samples per group in eWAT and rpWAT.
between treatment groups and between adipose tissue depots. Tukey’s post hoc comparisons were performed when necessary. A paired \( t \)-test was performed to test for significant differences between basal free glycerol release, FFA release, and FFA/glycerol ratio, between basal- and insulin-stimulated protein phosphorylation, and between ATOC treatment groups. Linear regression was performed to determine correlations between constitutive, unstressed HSP levels and basal glycerol release from ex vivo WAT explants, as well as FFA/glycerol. Where raw values did not meet the ANOVA assumptions of normally distributed data or equal variance, the raw values were transformed.

Fig. 2. Induction of HSPs in response to in vitro heat treatment. HSP72 (A), HSP25 (B), phospho-HSP25 (Ser-82) (C), and HSP60 (D) expression in white adipose tissue organ cultures (ATOCs) following a single in vitro heat treatment. Adipose tissue from SCAT, eWAT, and rpWAT were removed from anesthetized male Wistar rats, minced, and placed in separate culture dishes. HSP expression was determined by Western blotting and protein levels normalized to Ponceau staining. *\( P < 0.05 \), **\( P < 0.01 \), ***\( P < 0.001 \) denotes a significant increase following heat treatment determined by paired \( t \)-test. †\( P < 0.05 \) denotes that the induction of HSP expression is significantly greater in the SCAT compared with the eWAT and rpWAT. #\( P < 0.05 \) denotes that the induction of HSP expression is significantly greater in the SCAT compared with eWAT only determined by ANOVA. Values are mean fold changes ± SE. \( N = 5–6 \) samples per group.
logarithmically or by square root to meet these assumptions. Raw values are presented in all figures.

RESULTS

**Basal HSP expression.** We observed that HSP72 levels were greatest in eWAT, intermediate in rpWAT, and lowest in SCAT from sham-treated animals (eWAT and rpWAT: ~5.4-fold and ~2.9-fold greater than SCAT, respectively, and eWAT: ~1.9-fold greater than rpWAT) (Fig. 1A). Total HSP25 levels were ~5.8-fold greater and ~6.0-fold greater in the eWAT and rpWAT compared with SCAT, respectively (Fig. 1B). There were no differences in phospho-HSP25 (Ser-82) relative to HSP25 and in HSP60 between WAT depots (Fig. 1, C and D).

**Induction of HSPs by heat treatment.** HSP72 content was significantly increased in all three depots 24 h following in vivo heat treatment, and the induction of HSP72 was greatest in the SCAT (~6.8-fold increase) (Fig. 1A). Total HSP25 levels were increased in the eWAT and rpWAT, but not different in SCAT (Fig. 1B). There were no changes in phospho-HSP25 relative to total HSP25 following heat treatment (Fig. 1C). HSP60 levels were increased following heat treatment in the rpWAT, but unchanged in the other depots (Fig. 1D).

To determine if the observed differences in the induction of HSP expression across WAT depots were influenced by variable distribution of heat in vivo, we performed heat treatment on adipose tissue organ cultures. We removed adipose tissue from a subset of control rats, placed the depots in culture, and then exposed each depot to heat treatment in vitro. Similar to what was observed in vivo, HSP72 increased following heat treatment in all three depots with the greatest induction in HSP72 occurring in the SCAT (Fig. 2A). HSP25 expression significantly increased in all three depots (Fig. 2B) while phospho-HSP25 relative to total HSP25 was unchanged (Fig. 2C). HSP60 significantly increased following heat treatment in the SCAT and rpWAT, while in vivo only the rpWAT showed an induction in HSP60 with heat treatment (Fig. 2D). In vitro, the basal expression of HSPs was not significantly different between depots. Overall, the induction of HSP72 is most robust in SCAT both in vivo and in vitro, and the HSP induction patterns in rpWAT and eWAT were consistent between in vivo and in vitro experiments. Interestingly, the SCAT was more responsive to in vitro heat treatment with significant increases in both HSP25 and HSP60, a pattern not observed in vivo.

**The impact of heat treatment on lipolysis and fatty acid reesterification.** Twenty-four hours after a single in vivo heat treatment, circulating glucose, insulin, glycerol, NEFA, or leptin concentrations were not significantly altered (Table 1). When examining markers of lipolysis in individual depots, basal free glycerol release from ex vivo adipose tissue explants was greatest in eWAT, intermediate in rpWAT, and lowest in SCAT when expressed relative to total protein content (eWAT 0.054 ± 0.002 μmol·μg protein⁻¹·h⁻¹, rpWAT 0.042 ± 0.003 μmol·μg protein⁻¹·h⁻¹, SCAT 0.014 ± 0.001 μmol·μg protein⁻¹·h⁻¹, P < 0.01). In addition, basal glycerol release was significantly correlated with constitutive HSP72 (Fig. 3A) and HSP25 levels from sham-treated animals (Fig. 3B). There were no differences in FFA release across adipose depots (data not shown). Parallel lipolysis, basal FFA/glycerol was lowest in eWAT, intermediate in rpWAT, and greatest in SCAT from sham-treated animals (Figs. 4, A–C), indicative of greater fatty acid reesterification in eWAT, intermediate in rpWAT, and lowest in SCAT. We also observed that basal FFA/glycerol negatively correlated with constitutive HSP72 (Fig. 3C) and HSP25 levels (Fig. 3D), indicating that constitutive HSP72 and HSP25 levels in WAT were positively correlated with fatty acid reesterification.

Following a single heat treatment, fatty acid reesterification was changed in a depot-specific fashion. Basal FFA/glycerol in rpWAT was significantly increased following heat treatment, indicative of a decrease in fatty acid reesterification (Fig. 4A). Conversely, basal FFA/glycerol in SCAT was significantly reduced following heat treatment (Fig. 4C). There were no differences in fatty acid reesterification in eWAT following heat treatment (Fig. 4B). Relative rates of fatty acid reesterification paralleled the results indicated by FFA/glycerol, but were not significantly different between sham and heat treatments (Table 2). Following heat treatment, there were no changes in glycerol release in any of the adipose tissue depots examined. FFA release was increased following heat treatment in the rpWAT, but was not altered in eWAT or SCAT (data not shown).

We examined protein levels and phosphorylation of a number of lipolytic enzymes and enzymes involved in fatty acid reesterification, but did not observe differences between phosphorylated hormone sensitive lipase (HSL) (Ser-660), total HSL, total adipose triglyceride lipase (ATGL), phosphoenolpyruvate carboxylase (PEPCK), and pyruvate dehydrogenase 4 (PDK4) between sham- and heat-treated animals in any of the WAT depots examined.

**Insulin responsiveness following in vivo heat treatment.** Ex vivo adipose tissue explants from the rpWAT, eWAT, and SCAT removed 24 h following either in vivo heat or sham treatment were incubated with insulin (100 μU/ml) to determine insulin responsiveness. Phosphorylation of Akt (Ser-473) was significantly increased in the rpWAT and eWAT following insulin incubation, but was not significantly increased in SCAT (Fig. 5, A–C). Phosphorylation of AS-160 (Thr-642) was also significantly increased following insulin incubation in the rpWAT and eWAT, but not significantly increased in SCAT (Fig. 5, D–F). Furthermore, we observed that phosphorylation of Akt (Fig. 5B) and AS-160 (Fig. 5E) following insulin incubation was significantly greater in eWAT explants from heat-treated animals compared with eWAT explants from sham-treated animals, but was not significantly different in rpWAT (Fig. 5, A and D) or SCAT (Fig. 5, C and F).

Table 1. Blood measures following a single in vivo heat treatment

<table>
<thead>
<tr>
<th></th>
<th>Sham Treated</th>
<th>Heat Treated</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mg/dl</td>
<td>137.0 ± 3.7</td>
<td>133.2 ± 4.6</td>
<td>= 0.53</td>
</tr>
<tr>
<td>Insulin, ng/ml</td>
<td>1.3 ± 0.3</td>
<td>1.3 ± 0.3</td>
<td>= 0.96</td>
</tr>
<tr>
<td>Leptin, ng/ml</td>
<td>0.53 ± 0.13</td>
<td>0.57 ± 0.13</td>
<td>= 0.82</td>
</tr>
<tr>
<td>Glycerol, mmol/l</td>
<td>0.17 ± 0.04</td>
<td>0.22 ± 0.03</td>
<td>= 0.44</td>
</tr>
<tr>
<td>NEFA, mmol/l</td>
<td>0.37 ± 0.06</td>
<td>0.45 ± 0.05</td>
<td>= 0.31</td>
</tr>
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</table>

Values are means ± SE. NEFA, nonesterified fatty acids. N = 5–6 samples per group.
DISCUSSION

New data from the present study demonstrate depot-specific patterns of HSP expression with higher HSP72 and HSP25 expression in more metabolically active WAT depots (i.e., eWAT and rpWAT). In addition, an acute in vivo heat treatment induces a unique HSP response across WAT depots. In general, HSP72 and HSP25 are highly inducible in WAT depots, with the greatest induction of HSP72 occurring in the SCAT, a depot with the lowest constitutive expression of this protein. These results are for the most part recapitulated when

Fig. 3. Correlations between HSP72, HSP25, lipolysis, and fatty acid reesterification. Correlations between basal HSP72 (A and C) and HSP25 (B and D) expression levels from SCAT, eWAT, and rpWAT and basal free glycerol release (A and B) and basal FFA/glycerol (C and D) from ex vivo adipose tissue explants. Twenty-four hours following in vivo sham treatment, WAT depots were removed and protein expression measured by Western blot. Protein levels were normalized to β-actin protein levels. WAT from each depot was also used for ex vivo adipose tissue explants, and free glycerol release into the media were assayed using colorimetric assays and normalized to protein content (μmol·μg protein⁻¹·2 h⁻¹). Basal fatty acid reesterification was quantified as the ratio of glycerol release to FFA release (FFA/glycerol). ●, basal SCAT samples; ▲, basal rpWAT samples; ○, basal eWAT samples.

Fig. 4. Fatty acid reesterification from ex vivo adipose tissue explants following heat treatment. Fatty acid reesterification (A–C) from ex vivo adipose tissue explants removed from male Wistar rats 24 h following a single in vivo heat or sham treatment. Ex vivo adipose tissue explants from the retroperitoneal, epididymal, and subcutaneous WAT depots were removed for ex vivo assessment of free glycerol and FFA release to determine fatty acid reesterification. Fatty acid reesterification was quantified as the ratio of FFA release to glycerol release (FFA/glycerol). *P < 0.05 denotes that heat treatment was significantly different than sham treatment in untreated, basal explants determined by ANOVA. Values are means ± SE. N = 5–6 samples per group.
examined in vitro. While an association between HSP expression and oxidative capacity has been established in skeletal muscle, the relationship between HSP expression and adipose tissue metabolism is less clear. Variability in HSP expression and induction patterns further supports the growing body of literature delineating the function of various WAT depots in health and disease.

**HSP expression in the adipose tissue.** In the present study, we observed marked differences in the expression levels and induction of HSPs in response to heat treatment across adipose tissue depots. Constitutive HSP72 levels demonstrated the pattern of eWAT > rpWAT > SCAT, while HSP25 was similar with eWAT/rpWAT > SCAT. Differences in HSP72 and HSP25 expression across adipose tissue depots observed in the present study may involve differences in the regulation or expression of heat shock factor-1 (HSF-1), the primary transcriptional regulator of HSP72 and HSP25 (22, 23). The differences observed in HSP expression between adipose tissue depots are in agreement with other laboratories that have reported greater HSP content in more metabolically active WAT depots of mice and humans (26, 29). Perez-Perez et al. (26) reported that in humans HSP72, HSP25, and HSP90 expression levels were similar with eWAT/rpWAT and SCAT. Differences in HSP72 tissue depots. Constitutive HSP72 levels demonstrated the pattern of eWAT > rpWAT > SCAT, while HSP25 was similar with eWAT/rpWAT > SCAT. Differences in HSP72 and HSP25 expression across adipose tissue depots observed in the present study may involve differences in the regulation or expression of heat shock factor-1 (HSF-1), the primary transcriptional regulator of HSP72 and HSP25 (22, 23). The differences observed in HSP expression between adipose tissue depots are in agreement with other laboratories that have reported greater HSP content in more metabolically active WAT depots of mice and humans (26, 29). Perez-Perez et al. (26) reported that in humans HSP72, HSP25, and HSP90 expression levels were similar with eWAT/rpWAT and SCAT. Differences in HSP72

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**Table 2. Absolute rates of basal fatty acid reesterification**

<table>
<thead>
<tr>
<th>WAT Depot</th>
<th>Sham Treated</th>
<th>Heat Treated</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rpWAT, µmol-g tissue −1·2 h−1</td>
<td>7.10 ± 0.86</td>
<td>6.16 ± 0.45</td>
<td>0.358</td>
</tr>
<tr>
<td>eWAT, µmol-g tissue −1·2 h−1</td>
<td>11.96 ± 1.30</td>
<td>12.35 ± 1.17</td>
<td>0.826</td>
</tr>
<tr>
<td>SCAT, µmol-g tissue −1·2 h−1</td>
<td>1.52 ± 0.60</td>
<td>3.10 ± 0.73</td>
<td>0.134</td>
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</tbody>
</table>

Values are means ± SE. N = 5–6 samples per group. rpWAT, eWAT, and SCAT are retroperitoneal, epididymal, and subcutaneous white adipose tissue depots, respectively.

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**Fig. 5.** Insulin response following a single in vivo heat treatment. Phosphorylation of Akt and AS-160 in explants from the retroperitoneal (A and D), epididymal (B and E), and subcutaneous (C and F) WAT depots. Adipose tissue explants were removed from male Wistar rats 24 h following a single in vivo heat or sham treatment for ex vivo assessment of insulin responsiveness. Explants were incubated with water (−INS) or insulin (+INS) (100 µU/ml) for 2 h after which explant tissue was removed and frozen at −80°C. Phosphorylation of Akt (Ser-473), total Akt, and phosphorylation of AS-160 (Thr-642) were determined by Western blotting. Phospho-Akt protein levels were normalized total Akt protein levels, and phospho-AS-160 protein levels were normalized to β-actin protein levels. *P < 0.05, **P < 0.01, ***P < 0.001 denotes that insulin stimulated condition was greater than non-insulin stimulated condition determined by paired t-test. †P < 0.05 denotes that insulin-stimulated condition in adipose tissue explants from heat-treated animals was significantly greater than insulin-stimulated condition in explants from sham-treated animals determined by ANOVA. Values are means ± SE. N = 5–6 samples per group.
higher in omental adipose tissue compared with SCAT, in support of our findings. In another human study, Peinado et al. (25) did not find differences in HSP72 expression between SCAT and visceral adipose tissue, or differences between adipocytes isolated from these depots. These investigators also found significantly greater HSP72 expression in the stromal-vascular fraction (SVF) of SCAT compared with SVF of visceral adipose tissue (25). There are differences in adipose tissue depots between humans and rodents and this could account for the observed differences between our study and Peinado et al. (25). In addition, Peinado et al. examined lean but older subjects (50–70 yr of age) and both HSP expression and induction of the heat shock response have been shown to be decreased with age (11, 30).

Our findings clearly demonstrate that HSP expression patterns differ across adipose tissue depots, and that the response to heat treatment is depot-specific as well. Induction of HSP72 and HSP25 was robust in all depots both in vivo and in vitro, with the exception of SCAT where induction of HSP25 did not occur in vivo. Typically, HSP72 and HSP25 are inducible to heat and more directly involved in the tissue stress response, compared with HSP60, which is primarily a mitochondrial chaperone protein (4, 23). This is supported by our findings where induction of HSP60 occurred only in the rpWAT in vivo. Changes in phosphorylation of HSP25 were not observed either in vivo or in vitro in any of the adipose tissue depots examined, despite significant induction of total HSP25 protein with heat treatment. It could be that changes in phosphorylation of HSP25 are not evident at the 24 h time point utilized in the present study. Differences between adipose tissue depots in the sensitivity and response to temperature have been observed in other experimental situations. Recently the SCAT of mice has been shown to be more sensitive to cold exposure (10°C for 20 h) than eWAT, as measured by the induction of peroxisome proliferator-activated receptor gamma co-activator-alpha (PGC-1α) and uncoupling protein-1 (UCP-1) mRNA (41). Future studies are needed to determine the effects of age and obesity on HSP expression and induction, as well as identify which cells in the mixed cell population of adipose tissue underlie observed HSP expression patterns.

Effects of heat treatment on WAT metabolism. The effect of in vivo heat treatment on WAT metabolism has previously not been well described. Early work by Torlinska et al. (33) pointed towards a reduction in lipolysis shortly following heat treatment in rats. In the present study, we observed distinct changes in WAT metabolism between depots 24 h following a single heat treatment. We observed the most pronounced changes in the rpWAT where basal lipolysis was increased and fatty acid reesterification was decreased. In the SCAT, we observed an increase in basal fatty acid reesterification without changes in lipolysis. As a result of obesity and metabolic dysfunction, the SCAT loses some of its ability to store fatty acids. This results in fatty acid storage in abdominal WAT depots and organs (i.e., skeletal muscle) and further metabolic dysfunction (28). The increase in fatty acid reesterification observed here in the SCAT following heat treatment may be of metabolic benefit during pro-obesity and proinflammatory conditions to help promote proper fatty acid storage. In essence, the robust increase in HSP72 observed in this depot may result in anti-inflammatory effects which protect against obesity-induced adipose dysfunction. Furthermore, following heat stress, HSP72 localizes to the lipid droplet surface in isolated adipocytes (16) and heat stress has been shown to modulate lipid membrane integrity (34–36). These processes may help explain the observed changes in lipolysis and fatty acid reesterification without observed changes in enzyme levels. We also observed that following a single in vivo heat treatment, insulin responsiveness increased in eWAT, but this enhanced insulin responsiveness did not occur in rpWAT and SCAT. These new insulin data further highlights the depot-specific response to heat treatment. Together, the short-term changes in fatty acid metabolism observed following a single heat treatment may result in improved metabolic profile, an idea that could lead to new understanding of the adipose tissue stress response.

Conclusions. The results of the present study indicate that HSP levels are greater in more metabolically active WAT depots. The induction of HSP72 occurs in all WAT depots examined and is greatest following a single heat treatment in the SCAT. The induction of HSP25 occurs in the rpWAT and eWAT in vivo and in all WAT depots in vitro. A single heat treatment alters WAT lipolysis, fatty acid reesterification, and insulin responsiveness in a depot-specific fashion. Future studies are needed to determine the effects of chronic heat treatment and HSP induction on adipose tissue function and potential protection from diet-induced obesity.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: R.S.R., M.-S.B., J.L.W., and P.C.G. performed experiments; R.S.R., M.-S.B., J.L.W., and P.C.G. analyzed data; R.S.R., M.-S.B., D.C.W., and P.C.G. interpreted results of experiments; R.S.R. prepared figures; R.S.R. drafted manuscript; R.S.R., M.-S.B., D.C.W., and P.C.G. edited and revised manuscript; R.S.R., D.C.W., and P.C.G. conceived and designed of research; P.C.G. approved final version of manuscript.

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