Thermotherapy has Sexually Dimorphic Responses in APP/PS1 Mice

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Abstract

A thermoregulatory decline occurs with age due to changes in muscle mass, vasoconstriction, and metabolism that lowers core body temperature (Tc). Although lower Tc is a biomarker of successful aging, we have previously shown this worsens cognitive performance in the APP/PS1 mouse model of Alzheimer's disease (AD) [1]. We hypothesized that elevating Tc with thermotherapy would improve metabolism and cognition in APP/PS1 mice. From 6-12 months of age, male and female APP/PS1 and C57BL/6 mice were chronically housed at 23 or 30°C. At 12 months of age, mice were assayed for insulin sensitivity, glucose tolerance, and spatial cognition. Plasma, hippocampal, and peripheral (adipose, hepatic, and skeletal muscle) samples were procured postmortem and tissue-specific markers of amyloid accumulation, metabolism, and inflammation were assayed. Chronic 30°C exposure increased Tc in all groups except female APP/PS1 mice. All mice receiving thermotherapy had either improved glucose tolerance or insulin sensitivity, but the underlying processes responsible for these effects varied across sexes. In males, glucose regulation was influenced predominantly by hormonal signaling in plasma and skeletal muscle glucose transporter 4 expression, whereas in females, this was modulated at the tissue level. Thermotherapy improved spatial navigation in male C57BL/6 and APP/PS1 mice, with the later attributed to reduced hippocampal soluble amyloid-β $(A\beta)_{42}$. Female APP/PS1 mice exhibited worse spatial memory recall after chronic thermotherapy. Together, the data highlights the metabolic benefits of passive thermotherapy, but future studies are needed to determine therapeutic benefits for those with AD.

Introduction

Alzheimer's disease (AD) is characterized by a progressive deterioration in new learning and memory. This neurodegenerative disorder has multiple etiologies [2], with aging identified as the primary risk factor. Factors associated with normal aging may hasten disease progression. For example, basal metabolic rate declines with age due to deficits in muscle mass, vasoconstriction, glucose metabolism, insulin signaling and thermoregulation [3]. All of which leads to a reduction of core body temperature (Tc) [4].

Low Tc is a biomarker of longevity [5,6] and exposure to hypothermic environmental temperatures triggers adaptive metabolic enhancements. However, the advantageous aspects of lower Tc may adversely affect cognition. Mild hypothermia impairs cognitive performance in rats [7,8], nonhuman primates [9], and humans [10]. Alertness and cognition are strongly correlated with Tc whereby maximal mental performance is observed at higher temperatures [11]. Additionally, working, short-term, and long-term memory performance is decreased in humans during endogenous periods of lower Tc [11–13]. *In vitro* studies have demonstrated that amyloid fibril formation and tau hyperphosphorylation, both pathological hallmarks associated with AD, are accelerated at lower temperatures [14,15]. This potentially indicates a dualistic relationship between the mechanisms facilitating successful physiological aging and those contributing to pathological disease progression.

In our prior study, we demonstrated that APP/PS1 mice subjected to chronic 16˚C conditions exhibited improved insulin sensitivity. However, spatial learning and memory recall remained impaired, with females experiencing more pronounced deficits compared to littermates housed at ambient (23˚C) temperatures [1]. We also observed increased hippocampal plaque burden in male APP/PS1 mice, similar to observations in 3xTg mice when exposed to hypothermic

68 environmental temperature [16]. Alternatively, increasing Tc may be an effective strategy to treat
69 AD. Passive thermotherapy positively modulates health benefits across physical, cardiovascular,
70 and metabolic disorders [17] that are known AD risk factors [2].
71
72 We hypothesized that elevating Tc would enhance metabolism, thereby resulting in improved
73 learning and memory in the amyloidogenic APP/PS1 AD mouse model. Double transgenic
74 APP/PS1 mice express a chimeric mouse/human amyloid precursor protein (APP, Swe695) and
75 a mutant human presenilin (PS)1 lacking exon 9 (ΔE9). These mutations overexpress the amyloid
76 precursor protein with preferential cleavage of amyloid-β $(A\beta)_{42}$ isoforms [18]. Amyloid
77 accumulation and subtle cognitive impairments are observed at 6 months of age and become
78 prominent by 12 months [19]. We have previously shown APP/PS1 mice develop impairments in
79 insulin sensitivity and glucose homeostasis that contribute to their cognitive deficits [20]. Starting
80 at 6 months of age APP/PS1 and C57BL/6 littermate control mice were chronically housed in a
81 30°C controlled environmental chamber for 6 months. Translationally, this time frame corresponds
82 to conversion from MCI to AD, allowing us to determine the effects of mild hyperthermia on
83 metabolism and cognitive function during disease progression.

Results

Core Body Temperature: Tc was determined in a cohort of 11-12 month old mice maintained at ambient temperature since birth. Mice were then chronically exposed to thermotherapy (30˚C) for one month before Tc was measured. Thermotherapy increased Tc in all groups of mice except female APP/PS1 (Figure 1). The descriptive statistics for all bar graphs are shown in Table 1.

Thermotherapeutic effects on Blood Glucose: Repeated exposure (3-6 times per week) to thermotherapy for 30-60 minutes positively modulates insulin sensitivity and glucose tolerance in people with metabolic dysfunction [21,22]. Previous research shows APP/PS1 mice have reduced insulin sensitivity and glucose tolerance compared with age-matched littermate controls [1,20,23]. To determine thermotherapeutic effects on blood glucose regulation, we performed an insulin and glucose tolerance tests (ITT and GTT, respectively) after six months of chronic thermotherapy treatment. During the ITT, an intraperitoneal (ip) injection of insulin decreased blood glucose levels to a greater extent in all groups receiving thermotherapy, except for male APP/PS1, when compared with genotype and sex-matched ambient temperature controls (Figure 2A). The ITT area under the curve (AUC) provides an overall indication of insulin sensitivity. Thermotherapy improved insulin sensitivity in all groups apart from male APP/PS1 mice (Figure 2B-C). The GTT provides an indication of the endogenous uptake of blood sugar into tissue for energy utilization or storage. Thermotherapy exposed male APP/PS1 and female C57BL/6 mice receiving thermotherapy had lower blood glucose levels 15 minutes after an ip injection of glucose than their corresponding ambient temperature controls (Figure 2D). No time point differences were observed in male C57BL/6 or female APP/PS1 mice. The GTT AUC provides an indication of overall glucose tolerance which was only improved in female C57BL/6 mice receiving thermotherapy (Figure 2E-F). Thermotherapy worsened fed blood glucose in male C57BL/6

109 (Figure 2G) and fasting blood glucose in male APP/PS1 mice (Figure 2H) despite their improved
110 insulin sensitivity and glucose tolerance, respectively.

111 *Effects of glucose regulating plasma peptides after chronic thermotherapy:*
112 Circulating levels of plasma peptides and hormones that modulate blood glucose levels were
113 measured by multiplex assays. Glucagon release from the pancreas promotes liver
114 glycogenolysis thereby increasing circulating blood glucose levels. Chronic thermotherapy
115 reduced plasma glucagon levels in all groups except female C57BL/6 mice (Figure 3A).
116 Glucagon-like peptide 1 (GLP1) release from the gastrointestinal tract reduces circulating glucose
117 levels by stimulating insulin release, suppressing glucagon secretion, and promoting satiety.
118 Thermotherapeutic differences in plasma GLP1 levels were only observed in C57BL/6 mice with
119 decreased levels in males and an increase observed in females (Figure 3B). Despite the changes
120 observed in GLP1, thermotherapy did not affect plasma insulin in C57BL/6 mice, but a decrease
121 was observed in male APP/PS1 mice (Figure 3C). Fibroblast growth factor 21 (FGF21) regulates
122 metabolism and is important for thermogenic recruitment of white adipose tissue (WAT). Plasma
123 FGF21 was elevated in male C57BL/6 and APP/PS1 mice housed at 30˚C, but no differences
124 were observed in females receiving thermotherapy (Figure 3D). B-cell activating factor (BAFF) is
125 a member of the tumor necrosis factor (TNF) ligand family that regulates adipose tissue
126 inflammation and impairment of insulin-receptor signaling [24]. Chronic thermotherapy treatment
127 reduced plasma BAFF in both genotypes of male mice as well as female APP/PS1 mice, similar
128 to plasma glucagon observations (Figure 3E). Taken together, these results show that six months
129 of passive thermotherapy altered the plasma profile of peptides and hormones in a manner
130 consistent with improved insulin sensitivity and glucose tolerance in a sex-dependent manner.

131 *Hepatic glucose metabolizing genes:* The liver plays a key role in maintaining an easily accessible
132 supply of energy, primarily in the form of glucose and glycogen. Insulin receptor (InsR) activation
133 leads to downstream signal transduction through phosphatidylinositol 3-kinase (PI3K) / Akt that

134 induces translocation of glucose transporters to the cell surface, thereby facilitating glucose
135 uptake. Once in the liver, glucose undergoes phosphorylation by glucokinase (Gck) to form
136 glucose-6-phosphate, which can be utilized in either glycolysis or glycogenesis. Conversely,
137 glucose-6-phosphatase (G6PC) catalyzes the hydrolysis of glucose-6-phosphate back to glucose.
138 Chronic thermotherapy affected genes associated with liver glucose uptake in female C57BL/6
139 mice. InsR and Akt expression were similar, but PI3K relative gene expression was elevated in
140 after thermotherapy treatment (Figure 4A-C). This increased expression accounts for the
141 improved insulin sensitivity seen in the female C57BL/6 mice as previously discussed (Figure 2A,
142 C). No difference in Glut2 expression were observed (Figure 4D), but GCK and G6PC were both
143 decreased in female APP/PS1 mice receiving thermotherapy treatment (Figure 4E-F).
144 Conversely, thermotherapy increased G6PC expression in female C57BL/6 mice. This variation
145 in G6PC between female C57BL/6 and APP/PS1 mice likely contributes to their divergent
146 responses during the GTT. Following thermotherapy treatment, glucose tolerance was enhanced
147 in C57BL/6, but diminished in APP/PS1 mice.

148 *Perigonadal white adipose tissue mRNA expression:* Visceral adipose tissue is hormonally active
149 and regulates systemic metabolism by acting as an energy reservoir and through secretion of
150 adipokines and cytokines. Excessive accumulation of this adipose depot causes insulin
151 insensitivity and release of proinflammatory cytokines resulting in metabolic dysregulation.
152 Localized thermotherapy has been shown to induce browning of WAT through increased
153 expression of uncoupling protein (UCP) 1 [25]. UCP1 is present in brown and beige adipose
154 tissue and is responsible for dissipating the mitochondrial electron transport gradient to generate
155 heat instead of ATP synthesis. Chronic thermotherapy increased perigonadal (pg) WAT UCP1
156 expression in all groups except female C57BL/6 mice, where a decrease was observed (Figure
157 4G). Peroxisome proliferator-activated receptor-gamma coactivator (PGC)-1α regulates
158 expression of UCP1 along with FGF21 signaling. PGC-1α expression was upregulated in male

159 C57BL/6 mice after thermotherapy treatment (Figure 4H). This increased gene expression
160 coupled with FGF21 plasma concentrations (Figure 3D) indicates that thermotherapy increased
161 gene expression pathways implicated in the browning of adipose tissue. Thermotherapy
162 modulated InsR expression in female mice, but in opposing manners, with reduced expression in
163 C57BL/6 and an increase observed in APP/PS1 mice (Figure 4I). This would account for the
164 improved insulin sensitivity in female APP/PS1, but not sex-matched C57BL/6 mice.
165 Proinflammatory cytokines systemically impair glucose homeostasis. Similar to InsR expression,
166 TNFα and Interleukin (IL)-6 mRNA levels were divergent in female mice after thermotherapy
167 treatment. In females, expression of both proinflammatory cytokines were decreased in pgWAT
168 of C57BL/6, but increased in APP/PS1 mice (Figure 4J-K). The increased release of
169 proinflammatory cytokines from pgWAT in female APP/PS1 likely contributes to the worsening of
170 glucose tolerance discussed previously (Figure 2D,F).

171 *Skeletal Muscle Glucose Transporter Expression:* Skeletal muscle is the predominant tissue
172 responsible for blood glucose homeostasis mediated through insulin-dependent (Glut4) and
173 independent (Glut1) mechanisms. Under basal conditions, Glut1 is located on the plasma
174 membrane, whereas Glut4 resides intracellularly. In response to insulin or exercise, Glut4 is
175 translocated to the plasma membrane and is crucial for systemic glucose homeostasis. Glut1 can
176 also enhance basal glucose uptake in skeletal muscle [26]. Thermotherapy showed opposing
177 effects on Glut4 expression in male mice with decreases in C57BL/6 but increases in APP/PS1
178 mice (Figure 4L), while Glut1 expression was similar in both genotypes (Figure 4M). In male
179 APP/PS1 mice, Glut4 expression levels were elevated (although not significantly) that could
180 contribute to improving their glucose tolerance (Figure 2D). Whereas in male C57BL/6 mice,
181 decreased Glut4 expression after thermotherapy does not affect glucose tolerance despite
182 improvements in insulin signaling. In females, thermotherapy did not alter Glut4 but increased
183 expression of Glut1 in C57BL/6 mice (Figure 4L-M). This enhanced Glut1 expression in female

184 C57BL/6 would account for the improvement in glucose tolerance discussed earlier (Figure 2D),
185 particularly at the earliest time point.

186 *Thermotherapy alters spatial learning and memory in a sexually dimorphic manner:* Spatial
187 learning and memory recall is impaired in APP/PS1 mice by 12 months of age [1]. To determine
188 if thermotherapy ameliorates these cognitive deficits, we tested mice using the Morris water maze
189 (MWM) spatial navigation paradigm. During the training sessions, platform latency was
190 decreased in male C57BL/6 mice exposed to 30˚C (Figure 5A). All other groups had a similar
191 learning profile. During the probe challenge, the number of platform entries (Figure 5B) were
192 increased while latency to first platform entry (Figure 5C) was decreased in male C57BL/6 mice
193 after thermotherapy. Female APP/PS1 mice exhibited a decrease in the number of platform
194 entries and an increase in platform latency for first entry (Figure 5B-C). When examining an area
195 slightly larger than the former location of the hidden escape platform, the number of annulus 40
196 entries (Figure 5D) were increased in both male C57BL/6 and APP/PS1 mice receiving
197 thermotherapy. This also reduced the latency to first annulus 40 entry in male APP/PS1 mice
198 (Figure 5E), but an increased latency in female APP/PS1 mice was observed. Thermotherapy
199 increased the swimming speed in male C57BL/6 mice only (Figure 5F) that may be indicative of
200 skeletal muscle enhancements due to the warmer environment [27]. This faster swimming along
201 with an improved learning curve in male C57BL/6 mice accounts for their improved memory recall.
202 However, in AD mice, these performance issues were not observed. Thermotherapy has sexually
203 dimorphic cognitive effects regardless of AD genotype.

204 *Thermotherapy reduces hippocampal soluble $A\beta_{42}$ concentration in male APP/PS1 mice:* Soluble
205 $A\beta_{42}$ is considered to be a neurotoxic species and its aggregation of monomers, oligomers, and
206 protofibrils are associated with cognitive decline in AD [28]. Transgenic APP/PS1 mice
207 overexpress APP and PS1 resulting in preferential cleavage of the 42 base pair Aβ peptide. A
208 soluble $A\beta_{42}$ specific ELISA was used to measure hippocampal concentrations in APP/PS1 and

209 C57BL/6 mice, with the latter serving as a negative control (Figure 6). Thermotherapy reduced
210 the hippocampal $A\beta_{42}$ concentration in APP/PS1 male mice, but no effects were observed in
211 females of either genotype. This reduction of $A\beta_{42}$ in male APP/PS1 mice likely contributes to the
212 improved spatial memory recall.

213

214 **Discussion**

215 The age of the mice for this study was chosen based on the pathology and cognitive aspects of
216 disease progression. Amyloid accumulation and subtle cognitive impairments are observed at 6
217 months of age and rapidly progress to 12 months in APP/PS1 mice. This time frame translationally
218 corresponds to mild cognitive impairment which is the opportune window to initiate disease-
219 modifying treatment to prevent conversion to AD. Various biological and environmental factors
220 contribute to the onset and progression of AD, leading to the development of cognitive deficits as
221 well as metabolic and physical decline. To improve the overall quality of life for individuals with
222 AD, effective treatments should target a range of physiological changes beyond cognitive
223 impairments. Passive thermotherapy positively modulates multiple physiological parameters and
224 represents a nonpharmacological approach for potential disease modifying treatment.
225 Polypharmacy is common among aging populations, and nonpharmacological interventions help
226 mitigate adverse drug reactions.

227 Despite passive thermotherapy increasing Tc in both sexes of C57BL/6 mice, we observed some
228 notable differences between the sexes. Insulin sensitivity was improved in both sexes, but to a
229 much larger degree in the females. Improved glucose tolerance was only observed in female
230 C57BL/6 mice. Thermotherapy significantly influenced plasma hormone levels in male C57BL/6
231 mice, while mRNA expression levels were more affected in females. In male mice, plasma
232 glucagon, GLP1, and BAFF levels decreased, whereas FGF21 levels increased. In female mice,
233 only GLP1 levels showed an increase. The increased expression of hepatic PI3K, combined with
234 the decreased expression of INSR in pgWAT, likely contributed to the improved insulin sensitivity
235 observed in female C57BL/6 mice following thermotherapy treatment. Additionally, we observed
236 reduced expression levels of proinflammatory cytokines in the pgWAT of these female mice.
237 Finally, thermotherapy improved spatial learning and memory only in male C57BL/6 mice, but this

238 may be attributed to improved muscle performance since swimming speed was faster in these
239 mice.

240 Our present study shows that passive thermotherapy improved either glucose tolerance or insulin
241 sensitivity in all sexes and genotypes tested. The underlying processes responsible for these
242 effects are diverse and sexually dimorphic. In males, glucose regulation was influenced
243 predominantly by plasma hormone signaling and skeletal muscle Glut4 expression, whereas in
244 females, this was modulated at the level of individual tissues. Reduced plasma glucagon levels
245 decrease glycogenolysis, improved insulin sensitivity accompanies lower BAFF concentrations,
246 and skeletal muscle Glut4 expression regulates uptake of circulating blood glucose levels. An
247 increase in plasma FGF21 and elevated gene expression of UCP1 and PGC-1α in pgWAT
248 indicates thermotherapy induced WAT browning that improved metabolism, similar to previous
249 observations [25]. Female mice receiving thermotherapy treatment showed significantly
250 enhanced responses to insulin that were mediated by changes in plasma hormones and pgWAT
251 genes. In female C57BL/6 mice, thermotherapy enhanced insulin signaling and lowered blood
252 glucose levels. This was accompanied by elevated plasma concentrations of GLP1 and hepatic
253 PI3K gene expression. In thermotherapy treated female APP/PS1 mice, decreased BAFF plasma
254 concentrations and increased pgWAT InsR expression would also improve their response to
255 insulin. However, the elevated expression of proinflammatory cytokines in pgWAT after
256 thermotherapy would cause the slower glucose clearance in these mice.

257 Despite the metabolic improvements observed in both sexes after thermotherapy, cognitive
258 improvements were only observed in male mice. Soluble $A\beta_{42}$, rather than insoluble fibrils and
259 plaques, is considered neurotoxic due to its ability to spread throughout the brain and affect
260 energy metabolism, neurotransmission, and inflammation. This eventually causes synapse and
261 cell loss that is a contributing factor to cognitive decline. Soluble $A\beta_{42}$ was reduced in males of
262 both genotypes, consistent with their improved spatial navigation. Although thermotherapy did

263 not affect soluble $A\beta_{42}$ levels in female APP/PS1 mice, their memory recall was significantly
264 decreased. This could be attributed to the elevated proinflammatory cytokine gene expression
265 observed peripherally. In addition to affecting glucose tolerance, TNFα and IL-6 can readily cross
266 the blood brain barrier and exacerbate the neuroinflammation already caused by plaque
267 formation.

268 Both passive cooling and thermotherapy have been explored in different models of AD and have
269 shown varying results. Acute and chronic passive cooling negatively affected AD pathology and
270 cognition in the 3xTg [16] and the APP/PS1 [1] models of AD, respectively. In the 3xTg model,
271 repeated bouts of passive cooling reduced tau phosphorylation, but had no effect on amyloid
272 pathology. The cognitive implications of these findings were not determined [29]. Thermotherapy
273 reduced soluble $A\beta_{42}$ [16] and tau phosphorylation [30] while improving cognitive performance in
274 the 3xTg mice, which is similar to the male APP/PS1 mice assayed in the present study. Unlike
275 males, chronic thermotherapy in female APP/PS1 mice worsened cognitive performance similar
276 to findings in Tg2576 mice [31].

277 In our present study, the APP/PS1 female mice were the only group that did not experience an
278 increase in Tc after thermotherapy and, coincidentally, exhibited poorer performance on the MWM
279 paradigm compared with normothermic controls matched for genotype and sex. Despite Tc
280 modulation by estradiol and progesterone [32], the absence of a response is not solely attributed
281 to sex hormones, as thermotherapy increased Tc in female C57BL/6 mice. In mice,
282 thermoregulatory sex differences have not been fully elucidated, but females prefer warmer
283 environments than males regardless of gonadal factors [32] despite having higher Tc [33]. This
284 suggests the differential responses we observed in the present study were not driven by sex
285 hormones, but rather $A\beta_{42}$ accumulation and aggregation. Further research is needed to
286 determine how this accumulation might affect hypothalamic preoptic area modulation of Tc.

287 Humans and other mammals are homeothermic, able to maintain a stable Tc through their
288 metabolic activity, regardless of external environmental influences. Most people in developed
289 nations spend a majority of their time residing in temperature controlled environments that are
290 optimized for thermal comfort, but minimize thermogenesis [34]. This coupled with the
291 increasingly sedentary nature of work and life reduces activity-related heat production resulting in
292 thermostasis [35]. Accordingly, exposure to thermal extremes has gained scientific interest for its
293 numerous physiological benefits that could either reduce risk factors associated with AD onset or
294 be a treatment for disease progression [17]. Japanese ofuro and Scandinavian sauna bathing has
295 been used for centuries with the latter associated with reduced risk of dementia and AD [36].
296 Studies demonstrate that exercise and passive heating have comparable beneficial physiological
297 effects [37]. Hence, passive thermotherapy would be a preferable alternative to exercise,
298 especially in elderly and frail individuals.

299 A couple of limitations should be considered when interpreting the findings of this study. Protein
300 levels rather than gene expression would provide a more causal mechanistic underpinning to the
301 observed metabolic differences that were reported. In particular, mRNA expression does not infer
302 recruitment of glucose transporters to the plasma surface and this analysis would be a better
303 measure of changes in glucose tolerance. Finally, APP/PS1 females undergo reproductive
304 senescence at earlier ages than C57BL/6 mice which could influence glucose metabolism and
305 cognitive performance. To avoid these cofounds, we analyzed within genotype treatment
306 comparisons.

307 Our present research adds to a growing body of literature highlighting the benefits of passive
308 thermotherapy to modulate AD progression and cognition in male, but not female mice, despite
309 observing greater metabolic effects in the latter. While these sexually dimorphic responses have
310 not been fully elucidated, further research is needed to improve the translational applications,
311 such as investigating the duration of thermotherapy treatment and determining if similar effects

312 are observed regardless of disease severity or pathological progression. Both of these factors are
313 ongoing research efforts in our laboratory. The metabolic benefits highlighted in the present study
314 also have translatable implications to other research areas such as obesity, diabetes, or metabolic
315 syndrome. Finally, thermotherapy can be applied without causing drug-drug interactions and
316 could replace the health benefits of exercise in frailer individuals.

317 **Methods**
318
319 Animals: Male and female APP/PS1 and littermate control C57BL/6 mice used for this study were
320 bred and maintained in our animal colony and originated from founder C57BL/6J
321 (RRID:IMSR_JAX:000664) and APP/PS1 (RRID:MMRRC_034832-JAX) mice from Jackson
322 Laboratory (Bar Harbor, ME). A 5 mm tail tip was sent to TransnetYX®, Inc (Cordova, TN) to
323 confirm genotypes. Mice were group-housed according to sex and genotype on a 12:12 hour light
324 / dark cycle, and laboratory rodent diet (LabDiet, 5001) and water were available *ad libitum*. The
325 *in vivo* assays were performed in the same order (ITT, GTT, MWM) for all mice with a minimum
326 of one week apart to limit effects of stress. One week post cognitive assessment, mice were
327 deeply anesthetized with isoflurane and a cardiac puncture for blood chemistry analysis was
328 performed. Immediately following, mice were euthanized by decapitation. Tissues were extracted
329 and stored at -80°C until processing.
330
331 Chemicals: Unless otherwise noted, all chemicals were prepared and stored according to
332 manufacturer recommendations.
333
334 Thermotherapy: From 6 to 12 months of age, mouse cages were placed into an environmental
335 chamber (Powers Scientific Cat: RIS33SD) maintained at 30 ± 1°C located within the same animal
336 facility room. Mice were only removed from this chamber for cage cleanings. A separate cohort
337 of age- and sex-matched C57BL/6 and APP/PS1 mice were maintained at a standard animal
338 room ambient temperature (23 ± 1°C) and used as a within genotype temperature control.
339
340 Core Body Temperature Measurements: A separate cohort of male and female C57BL/6 and
341 APP/PS1 mice were maintained at standard ambient temperature from birth until 11 months of
342 age. A rodent thermometer with rectal probe (TK8851; BioSebLab) was used to obtain Tc when
343 mice were 11 months old. These mice were then transferred to environmental chambers (Powers

344 Scientific Cat: RIS33SD) maintained at 30˚C until 12 months of age (1 month chronic treatment).
345 Mice were transferred to a separate room within our animal facility maintained at 30˚C to assess
346 changes in Tc after one month of chronic exposure.
347
348 Intraperitoneal (ip) Insulin Tolerance test (ITT) and Glucose Tolerance Test (GTT): To determine
349 insulin sensitivity, an initial blood glucose measurement (time = 0) was taken from the tail vein of
350 fed mice and measured using a Presto® glucometer (AgaMatrix, Salem, NH) followed by ip
351 injection of 1 IU / kg bw Humulin® R (Henry Schein, Melville, NY: Cat: 1238578). To determine
352 glucose tolerance, an initial blood glucose measurement was taken (time = 0) from fifteen hour
353 fasted mice followed by an ip injection of 2 g of dextrose / kg bw (Fisher Scientific Cat: D15).
354 Following either injection, blood glucose levels were measured sequentially at 15, 30, 45, 60, and
355 120 min [20].
356
357 Morris Water Maze (MWM) Training and Probe Challenge: At approximately 12 months of age,
358 mice underwent cognitive assessment using the MWM spatial learning and memory recall
359 paradigm, during which mice are trained to utilize visual cues to repeatedly swim to a static,
360 submerged hidden platform. The MWM paradigm consisted of 5 consecutive learning days with
361 three, 90-sec trials/day and a minimum 20 minute inter-trial interval. During the delayed memory
362 recall, the platform was removed and mice were given a single, 60 second probe challenge. The
363 ANY-maze video tracking system (Stoelting Co., Wood Dale, IL; RRID:SCR_014289) was used
364 to record navigational parameters and data analysis. The three trials for each training day were
365 averaged for each mouse for analysis. Variables extracted from ANY-maze and utilized for data
366 analysis include platform entries and latency, annulus 40 entries and latency, and swimming
367 speed.
368

369 Blood Chemistry: A cardiac puncture was used to collect blood in EDTA coated tubes (Sarstedt
370 Inc. Microvette CB 300) on wet ice until centrifugation at 1500 x g for 10 min at 4°C. The plasma
371 supernatant was collected and stored at -80°C until analysis with a multiplex assay kit (Meso
372 Scale Discovery) according to the manufacturer's recommended protocols.

373 RT-PCR: RNA was extracted from tissue and quantified using a NanoDrop One
374 spectrophotometer (Thermo Fisher Scientific) according to our previously published protocols [1].
375 cDNA was synthesized using candidate primers (Integrated DNA Technologies; Table 2) and an
376 iScript cDNA Synthesis Kit (Bio-Rad). Relative mRNA expression was analyzed by quantitative
377 RT-PCR using the QuantStudio PCR System (Applied Biosystems) and SYBR Green MasterMix
378 (Bio-Rad). Beta-2-microglobulin (B2M) was used as the internal housekeeping gene for pgWAT
379 and liver while GAPDH was used for skeletal muscle.

380 Soluble $A\beta_{42}$ Determination: The hippocampus from one hemisphere was dissected and stored
381 at -80°C until tissue processing. Soluble $A\beta_{42}$ concentrations were determined using the Human
382 / Rat β amyloid ELISA kits (WAKO Chemicals; Cat: 292-64501) according to the manufacturer
383 recommended protocols.

384 Statistical Analysis: Prism software Version 10.2 (GraphPad Software, Inc., La Jolla, CA;
385 RRID:SCR_002798) was used for all statistical analyses. A two-way ANOVA was used to test
386 for significance of temperature within a genotype for the ITT, GTT, and MWM learning assays.
387 Temperature treatment differences within the same sex and genotype were determined using a
388 two-tailed Student's t-test for all remaining assays. Potential outliers were determined with a
389 single Grubb's test (α=0.05). Data are represented as mean ± SEM and significance was defined
390 as $p<0.05$.

391 **Abbreviations**

392 Aβ (amyloid-β), AD (Alzheimer's disease), AUC (area under the curve), B2M (beta-2-
393 microglobulin), BAFF (B-cell activating factor), FGF21 (fibroblast growth factor 21), core body
394 temperature (Tc), G6PC (glucose 6-phosphatase), Gck (glucokinase), GLP1 (glucagon-like
395 peptide 1), Glut (glucose transporter), GTT (glucose tolerance test), InsR (insulin receptor), ip
396 (intraperitoneal), ITT (insulin tolerance test), MWM (Morris water maze), perigonadal (pg) PGC
397 (peroxisome proliferator-activated receptor-gamma coactivator), PI3K (phosphatidylinositol 3-
398 kinase), Tc (core body temperature), TNF (tumor necrosis factor), UCP (uncoupling protein), VAT
399 (visceral adipose tissue), WAT (white adipose tissue).

400

401 **Author Contributions**

402

403 SAM, MRP, LNS, MFC, EDI, CAF, KQ, and YF assisted with colony maintenance and the
404 experimental assays performed in this manuscript. AB and ERH assisted with experimental
405 design and manuscript revisions. KNH conceived the study, supervised the experiments,
406 analyzed the data, and wrote the manuscript. All authors approved the final version of the
407 manuscript.

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410 **Conflict of Interest**

411 The authors declare no conflicts of interest.

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417 and the Geriatrics Research Initiative (AB).

418 **Ethical Statement**

419 Protocols for animal use were approved by the *Institutional Animal Care and Use Committee* at
420 Southern Illinois University School of Medicine (IRB #2022-055) and in accordance with the
421 ARRIVE guidelines.

422

References

1. McFadden S, Sime LN, Cox MF, Findley CA, Peck MR, Quinn K, Fang Y, Bartke A, Hascup ER, Hascup KN. Chronic, Mild Hypothermic Environmental Temperature Does Not Ameliorate Cognitive Deficits in an Alzheimer's Disease Mouse. Anderson RM, editor. The Journals of Gerontology: Series A [Internet]. J Gerontol A Biol Sci Med Sci; 2022 [cited 2023 Jan 24]; . Available from: https://academic.oup.com/biomedgerontology/advance-article/doi/10.1093/gerona/glac223/6832816

2. Hascup ER, Hascup KN. Toward refining Alzheimer's disease into overlapping subgroups. Alzheimer's & Dementia: Translational Research & Clinical Interventions. John Wiley & Sons, Ltd; 2020; 6: 1–10.

3. Basu R, Breda E, Oberg AL, Powell CC, Man CD, Basu A, Vittone JL, Klee GG, Arora P, Jensen MD, Toffolo G, Cobelli C, Rizza RA. Mechanisms of the age-associated deterioration in glucose tolerance: Contribution of alterations in insulin secretion, action, and clearance. Diabetes. American Diabetes Association Inc.; 2003; 52: 1738–48.

4. Waalen J, Buxbaum JN. Is Older Colder or Colder Older? The Association of Age With Body Temperature in 18,630 Individuals. J Gerontol A Biol Sci Med Sci [Internet]. Oxford Academic; 2011 [cited 2021 Feb 25]; 66A: 487–92. Available from: https://academic.oup.com/biomedgerontology/article-lookup/doi/10.1093/gerona/glr001

5. Roth GS, Lane MA, Ingram DK, Mattison JA, Elahi D, Tobin JD, Muller D, Metter EJ. Biomarkers of caloric restriction may predict longevity in humans. Science. Science; 2002. p. 811.

6. Keil G, Cummings E, de Magalhães JP. Being cool: how body temperature influences ageing and longevity. Biogerontology [Internet]. Springer Netherlands; 2015 [cited 2019 Dec 3]; 16: 383–97. Available from: http://link.springer.com/10.1007/s10522-015-9571-2

7. Rauch TM, Welch DI, Gallego L. Hypothermia impairs performance in the Morris water maze. Physiol Behav. Elsevier; 1989; 46: 315–20.

8. Panakhova E, Burešová O, Bure J. The effect of hypothermia on the rat's spatial memory in the water tank task. Behav Neural Biol. Academic Press; 1984; 42: 191–6.

9. Bauer RH, Fuster JM. Delayed matching and delayed response deficit from cooling dorsolateral prefrontal cortex in monkeys. J Comp Physiol Psychol. 1976; 90: 293–302.

10. Coleshaw SRK, van Someren RNM, Wolff AH, Davis HM, Keatinge WR. Impaired memory registration and speed of reasoning caused by low body temperature. J Appl Physiol Respir Environ Exerc Physiol. 1983; 55: 27–31.

11. Wright KP, Hull JT, Czeisler CA. Relationship between alertness, performance, and body temperature in humans. Am J Physiol Regul Integr Comp Physiol. American Physiological Society; 2002; 283.

12. Johnson MP, Duffy JF, Dijk DJ, Ronda JM, Dyal CM, Czeisler CA. Short-term memory, alertness and performance: a reappraisal of their relationship to body temperature. J Sleep Res. John Wiley & Sons, Ltd; 1992; 1: 24–9.

13. Schmidt C, Collette F, Cajochen C, Peigneux P. A time to think: Circadian rhythms in human cognition. Cogn Neuropsychol. Psychology Press ; 2007; 24: 755–89.

14. Cohen SIA, Cukalevski R, Michaels TCT, Šarić A, Törnquist M, Vendruscolo M, Dobson CM, Buell AK, Knowles TPJ, Linse S. Distinct thermodynamic signatures of oligomer generation in the aggregation of the amyloid-β peptide. Nat Chem. Nature Publishing Group; 2018; 10: 523–31.

15. Carrettiero DC, Santiago FE, Motzko-Soares ACP, Almeida MC. Temperature and toxic Tau in Alzheimer's disease: new insights. Temperature. Routledge; 2015; 2: 491–8.

16. Vandal M, White PJ, Tournissac M, Tremblay C, St-Amour I, Drouin-Ouellet J, Bousquet M, Traversy M-T, Planel E, Marette A, Calon F. Impaired thermoregulation and beneficial effects of thermoneutrality in the 3×Tg-AD model of Alzheimer's disease. Neurobiol Aging [Internet]. 2016 [cited 2017 Oct 3]; 43: 47–57. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0197458016300082

17. Hunt AP, Minett GM, Gibson OR, Kerr GK, Stewart IB. Could Heat Therapy Be an Effective Treatment for Alzheimer's and Parkinson's Diseases? A Narrative Review. Frontiers in Physiology. Frontiers Media S.A.; 2020. p. 1556.

18. Jankowsky JL, Fadale DJ, Anderson J, Xu GM, Gonzales V, Jenkins NA, Copeland NG, Lee MK, Younkin LH, Wagner SL, Younkin SG, Borchelt DR. Mutant presenilins specifically elevate the levels of the 42 residue β-amyloid peptide in vivo: evidence for augmentation of a 42-specific γ secretase. Hum Mol Genet. Oxford University Press; 2004; 13: 159–70.

19. Webster SJ, Bachstetter AD, Nelson PT, Schmitt FA, Van Eldik LJ. Using mice to model Alzheimer's dementia: an overview of the clinical disease and the preclinical behavioral changes in 10 mouse models. Front Genet. 2014; 5: 1–14.

20. Hascup ER, Broderick SO, Russell MK, Fang Y, Bartke A, Boger HA, Hascup KN. Diet-induced insulin resistance elevates hippocampal glutamate as well as VGLUT1 and GFAP expression in AβPP/PS1 mice. J Neurochem. 2019; 148.

21. Hooper PL. Hot-Tub Therapy for Type 2 Diabetes Mellitus. New England Journal of Medicine. New England Journal of Medicine (NEJM/MMS); 1999; 341: 924–5.

22. Ely XBR, Clayton ZS, McCurdy XCE, Pfeiffer J, Needham KW, Comrada LN, Minson CT. Heat therapy improves glucose tolerance and adipose tissue insulin signaling in polycystic ovary syndrome. Am J Physiol Endocrinol Metab. American Physiological Society; 2019; 317: E172–82.

23. Macklin L, Griffith CM, Cai Y, Rose GM, Yan X-X, Patrylo PR. Glucose tolerance and insulin sensitivity are impaired in APP/PS1 transgenic mice prior to amyloid plaque pathogenesis and cognitive decline. Exp Gerontol. 2017; 88: 9–18.

24. Nakamura Y, Abe M, Kawasaki K, Miyake T, Watanabe T, Yoshida O, Hirooka M, Matsuura B, Hiasa Y. Depletion of B cell-activating factor attenuates hepatic fat accumulation in a murine model of nonalcoholic fatty liver disease. Sci Rep. Nature Publishing Group; 2019; 9: 1–12.

25. Li Y, Wang D, Ping X, Zhang Y, Zhang T, Wang L, Jin L, Zhao W, Guo M, Shen F, Meng M, Chen X, Zheng Y, et al. Local hyperthermia therapy induces browning of white fat and treats obesity. Cell. Elsevier B.V.; 2022; 185: 949-966.e19.

26. Gulve EA, Ren JM, Marshall BA, Gao J, Hansen PA, Holloszy JO, Mueckler M. Glucose transport activity in skeletal muscles from transgenic mice overexpressing GLUT1: Increased basal transport is associated with a defective response to diverse stimuli that activate GLUT4. Journal of Biological Chemistry. Elsevier; 1994; 269: 18366–70.

27. Kim K, Monroe JC, Gavin TP, Roseguini BT. REVIEW Physiology of Thermal Therapy: Skeletal muscle adaptations to heat therapy. J Appl Physiol [Internet]. American Physiological Society; 2020 [cited 2024 Aug 6]; 128: 1635–42. Available from: https://journals.physiology.org/doi/10.1152/japplphysiol.00061.2020

28. Walsh DM, Klyubin I, Fadeeva J V., Cullen WK, Anwyl R, Wolfe MS, Rowan MJ, Selkoe DJ. Naturally secreted oligomers of amyloid β protein potently inhibit hippocampal long-term potentiation in vivo. Nature. Nature Publishing Group; 2002; 416: 535–9.

29. Tournissac M, Bourassa P, Martinez-Cano RD, Vu TM, Hébert SS, Planel E, Calon F. Repeated cold exposures protect a mouse model of Alzheimer's disease against cold-induced tau phosphorylation. Mol Metab. Elsevier GmbH; 2019; 22: 110–20.

30. Guisle I, Pétry S, Morin F, Kérauden R, Whittington RA, Calon F, Hébert SS, Planel E. Sauna-like conditions or menthol treatment reduce tau phosphorylation through mild hyperthermia. bioRxiv. Cold Spring Harbor Laboratory; 2021; : 2021.01.27.428475.

31. Jung CG, Kato R, Zhou C, Abdelhamid M, Shaaban EIA, Yamashita H, Michikawa M. Sustained high body temperature exacerbates cognitive function and Alzheimer's disease-related pathologies. Sci Rep. Nature Research; 2022; 12: 1–11.

32. Kaikaew K, Steenbergen J, Themmen APN, Visser JA, Grefhorst A. Sex difference in thermal preference of adult mice does not depend on presence of the gonads. Biol Sex Differ [Internet]. BioMed Central Ltd.; 2017 [cited 2024 Mar 3]; 8: 24. Available from: http://bsd.biomedcentral.com/articles/10.1186/s13293-017-0145-7

33. Fernández-Peña C, Reimúndez A, Viana F, Arce VM, Señarís R. Sex differences in thermoregulation in mammals: Implications for energy homeostasis. Frontiers in Endocrinology. Frontiers Media S.A.; 2023. p. 1093376.

34. Moellering DR, Smith DL, Jr. Ambient Temperature and Obesity. Curr Obes Rep [Internet]. NIH Public Access; 2012 [cited 2019 Feb 27]; 1: 26–34. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24707450

35. Tipton M. Humans: A homeothermic animal that needs perturbation? Exp Physiol [Internet]. Blackwell Publishing Ltd; 2019 [cited 2024 Feb 27]; 104: 1–2. Available from: https://physoc.onlinelibrary.wiley.com/doi/10.1113/EP087450

36. Laukkanen T, Kunutsor S, Kauhanen J, Laukkanen JA. Sauna bathing is inversely associated with dementia and Alzheimer's disease in middle-aged Finnish men. Age Ageing [Internet]. Oxford University Press; 2017 [cited 2021 Mar 8]; 46: 245–9. Available from: https://academic.oup.com/ageing/article/46/2/245/2654230

37. Cullen T, Clarke ND, Hill M, Menzies C, Pugh CJA, Steward CJ, Thake CD. The health benefits of passive heating and aerobic exercise: To what extent do the mechanisms overlap? Journal of Applied Physiology. American Physiological Society; 2020. p. 1304–9.

578 Table 1: Descriptive statistics for all bar graphs.

Figure (units)	Male C57BL/6 23˚C	Male C57BL/6 30˚C	Male APP/PS1 23˚C	Male APP/PS1 30˚C	Female C57BL/6 23˚C	Female C57BL/6 30˚C	Female APP/PS1 23˚C	Female APP/PS1 30˚C
1 (˚C)	36.5±0.2	37.1±0.2*	36.5±0.2	37.1±0.1**	36.7±0.2	37.4±0.2**	36.8±0.2	37.1±0.2
2B-C (AUC)	10389±394	8834±507*	11432±524	11137±531	9163±268	6662±416****	10283±817	8172±369*
2E-F (AUC)	8159±519	8296±904	8723±744	7540±1025	7607±931	4786±595*	6337±1116	8541±962
2G (mg/dL)	176.1±4.7	214.3±9.9**	191.5±10.8	174.2±10.4	153.2±5.3	148.3±5.8	172.7±10.0	162.6±6.8
2H (mg/dL)	138.4±4.8	134.7±5.9	130.8±4.2	155.6±7.9*	127.3±5.7	128.3±5.5	154.1±10.7	131.7±3.3
3A (pM)	7.3±0.7	4.4±0.5**	7.9±1.0	4.7±0.7*	11.10±1.9	9.8±1.3	25.2±8.6	10.2±1.4
3B (pM)	23.1±2.5	12.0±0.8**	30.3±4.6	23.3±2.7	15.7±1.7	27.1±1.6***	27.88±4.9	25.9±4.0
3C (µIU/mL)	564.5±94.9	380.4±83.5	536.9±94.9	254.1±52.6*	375.0±92.4	370.9±96.7	236.3±43.8	385.7±130.4
3D (pg/mL)	86.1±10.7	192.1±42.9*	67.1±10.1	140.9±28.9*	119.7±46.5	86.1±12.1	135.5±45.0	77.2±7.5
3E (pg/mL)	2218±253	1197±156**	2340±154	1618±149**	1776±75	1521±96	3400±362	1683±265**
4A (RE)	1.0±0.2	1.5±0.2	1.0±0.2	0.7±0.1	1.0±0.1	1.0±0.2	1.0±0.1	0.8±0.1
4B (RE)	1.0±0.1	1.3±0.2	1.0±0.1	1.1±0.1	1.0±0.1	1.6±0.1**	1.0±0.1	0.8±0.1
4C (RE)	1.0±0.1	0.9±0.1	1.0±0.1	1.3±0.2	1.0±0.1	1.3±0.1	1.0±0.1	1.1±0.1
4D (RE)	1.0±0.2	1.1±0.2	1.0±0.3	1.3±0.3	1.0±0.1	0.7±0.2	1.0±0.2	1.1±0.1
4E (RE)	1.0±0.2	1.2±0.2	1.0±0.1	1.0±0.2	1.0±0.1	1.0±0.1	1.0±0.1	0.6±0.1*
4F (RE)	1.0±0.2	1.0±0.2	1.0±0.1	0.8±0.1	1.0±0.2	1.6±0.1*	1.0±0.2	0.6±0.1*
4G (RE)	1.0±0.4	2.8±0.9	1.0±0.3	2.2±0.7	1.0±0.3	0.2±0.1*	1.0±0.1	2.2±0.4*
4H (RE)	1.0±0.4	4.6±1.5*	1.0±0.3	1.4±0.4	1.0±0.3	1.1±0.4	1.0±0.4	1.1±0.2
4I (RE)	1.0±0.3	0.6±0.1	1.0±0.1	1.5±0.4	1.0±0.3	0.4±0.1*	1.0±0.1	2.6±0.5**
4J (RE)	1.0±0.4	2.2±0.7	1.0±0.2	1.6±0.6	1.0±0.2	0.3±0.2*	1.0±0.2	2.5±0.4**
4K (RE)	1.0±0.4	2.7±0.8	1.0±0.3	2.3±0.9	1.0±0.3	0.2±0.1*	1.0±0.2	1.8±0.3*
4L (RE)	1.0±0.2	0.4±0.1*	1.0±0.2	2.9±1.6	1.0±0.2	1.6±0.5	1.0±0.3	1.7±0.6
4M (RE)	1.0±0.2	1.1±0.2	1.0±0.1	1.0±0.3	1.0±0.1	1.8±0.1***	1.0±0.3	2.0±0.5
5B (entries)	2.0±0.4	3.2±0.4*	1.5±0.4	2.0±0.3	2.6±0.5	2.9±0.5	2.2±0.4	0.9±0.3*
5C (s)	31.3±5.9	15.3±2.3*	33.7±6.5	21.1±5.4	19.4±5.6	22.2±4.7	17.8±4.1	42.0±7.3**
5D (entries)	4.60±0.6	6.2±0.4*	3.2±0.7	5.2±0.6*	5.9±0.7	7.4±0.7	4.1±0.4	3.4±0.6
5E (s)	12.4±3.0	9.4±1.4	22.1±6.0	6.3±1.7*	7.7±1.5	11.4±2.6	7.3±1.5	17.7±3.9*
5F (m/s)	0.17±0.01	0.19±0.01*	0.17±0.01	0.19±0.01	0.20±0.01	0.20±0.01	0.21±0.01	0.21±0.01
6 (pmol/L)	15.6±3.2	7.4±2.8	482.4±58.2	224.5±65.3*	9.3±1.5	6.2±2.8	294.3±39.5	344.8±53.6

579 Values represent mean ± SEM rounded to the nearest whole number, tenth, or hundredth. *$p<0.05$, **$p<0.01$, ***$p<0.001$,
580 ****$p<0.0001$ indicate statistically significant differences from 23˚C sex- and genotype-matched controls. Abbreviations: area under
581 the curve (AUC), relative expression (RE)

582 Table 2: A list of forward and reverse mRNA primers.

Gene	Forward	Reverse
AKT1	5'-ATGAACGACGTAGCCATTGTG-3'	5'-TTGTAGCCAATAAAGGTGCCA-3'
B2M	5'-AAGTATACTCACGCCACCCA-3'	5'-AGGACCAGTCCTTGCTGAAG-3'
G6PC	5'-AGCTACATAGGAATTACGGGCAA-3'	CACAGTGGACGACATCCGAAA-3'
GAPDH	5'-AGGTCGGTGTGAACGGATTTG-3'	5'-TGTAGACCATGTAGTTGAGGTCA-3'
GCK	5'-TGAGCCGGATGCAGAAGG-3'	5'-GCAACATCTTTACACTGGCCT-3'
GLUT1	5'-TCAAACATGGAACCACCGCTA-3'	5'-AAGAGGCCGACAGAGAAGGAA-3'
GLUT2	5'-TCAGAAGACAAGATCACCGGA-3'	5'-GCTGGTGTGACTGTAAGTGGG-3'
GLUT4	5'-ACACTGGTCCTAGCTGTATTCT-3'	5'-CCAGCCACGTTGCATTGTA-3'
IL6	5'-CTGCAAGAGACTTCCATCCAG-3'	5'-AGTGGTATAGACAGGTCTGTTGG-3'
INSR	5'-CCTGGTTATCTTCGAGATGGTCC-3'	5'-CCCCACATTCCTCGTTGTCA-3'
PI3K	5'-TAGCTGCATTGGAGCTCCTT-3'	5'-TACGAACTGTGGGAGCAGAT-3'
PPARGC1A	5'-TATGGAGTGACATAGAGTGTGCT-3'	5'-GTCGCTACACCACTTCAATCC-3'
TNFA	5'-CAGGCGGTGCCTATGTCTC-3'	5'-CGATCACCCCGAAGTTCAGTAG-3'
UCP1	5'-AGGCTTCCAGTACCATTAGGT-3'	5'-CTGAGTGAGGCAAAGCTGATTT-3'

583 Abbreviations: Akt1 (serine / threonine kinase family), B2M (beta-2-microglobulin), G6PC
584 (glucose 6-phosphatase), GAPDH (glyceraldehyde 3-phosphate dehydrogenase), Gck
585 (glucokinase), Glut (glucose transporter), IL (interleukin), INSR (insulin receptor), PPARGC1A
586 (peroxisome proliferator-activated receptor-gamma coactivator), PI3K (phosphatidylinositol 3-
587 kinase), TNFA (tumor necrosis factor), UCP (uncoupling protein)
588

589 **Figure Legends**

590 **Figure 1: Effects of thermotherapy on Tc.**

591 Rectal temperature was determined before (23˚C) and after one month of thermotherapy
592 treatment (30˚C). The number of animals is inset on each bar graph. A two-tailed t-test was used
593 to determine changes in Tc within a genotype. *p<0.05, **p<0.01.

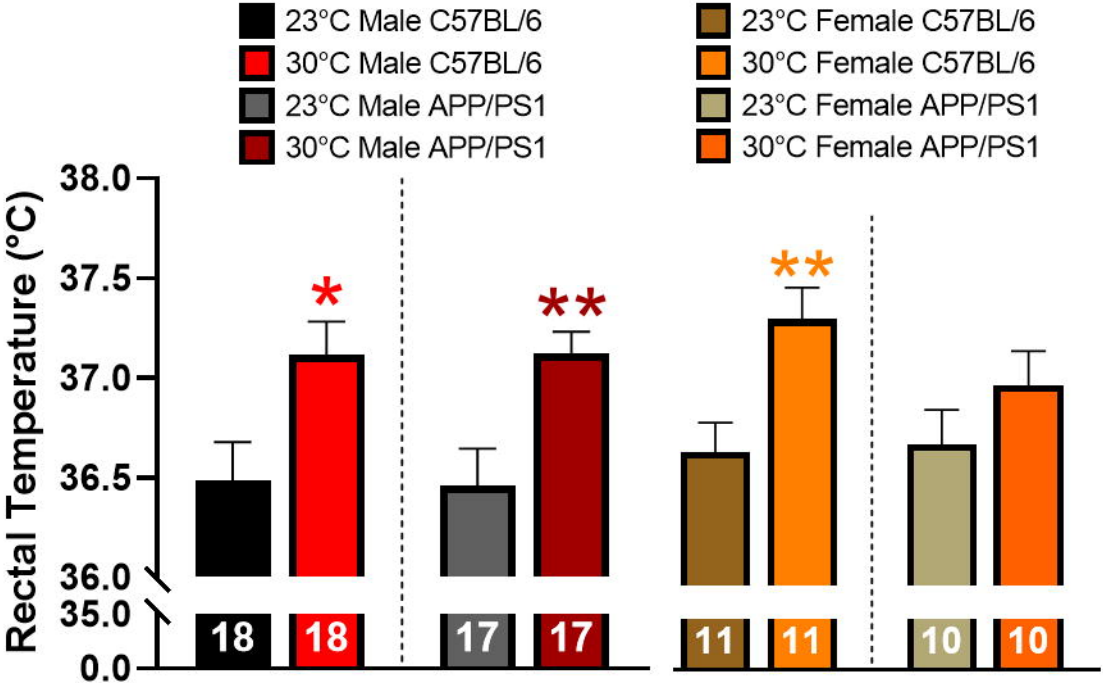
594 **Figure 2: Blood glucose changes after thermotherapy.**

595 Percent change of blood glucose levels after an ip injection of 1 IU / kg bw of insulin at t=0 minutes
596 (A). The AUC was calculated for the duration of the ITT and compared temperature effects in
597 male (B) and female (C) mice. Percent change of blood glucose levels after an ip injection of 2 g
598 / kg bw of glucose at t=0 minutes (D). The AUC was calculated for both male (E) and female (F)
599 mice. Fed (G) and fasting (H) blood glucose levels were determined prior to ip injection of insulin
600 and glucose, respectively. The number of animals is inset on each bar graph. A two-way ANOVA
601 factor analysis with Sidak's post hoc was used to determine significant blood glucose changes
602 due to thermotherapy across time intervals. A two-tailed t-test was used to determine blood
603 glucose changes within a genotype and sex. *p<0.05, **p<0.01, ****p<0.0001.

604 **Figure 3: Plasma concentrations of glucose regulating hormones.**
605 Plasma expression levels of glucagon (A), glucagon-like peptide 1 (GLP1; B), insulin (C),
606 fibroblast growth factor 21 (FGF21; E), and B-cell activating factor (BAFF; E) as detected by
607 multiplex assay. A two-tailed t-test was used to determine plasma concentration changes within
608 a genotype and sex. *p<0.05, **p<0.01, ***p<0.001.
609

610 **Figure 4: Hepatic, perigonadal white adipose tissue, and skeletal muscle mRNA**
611 **expression.**

612 Hepatic mRNA expression of insulin receptor (INSR; A), phosphatidylinositol 3-kinase (PI3K; B),
613 AKT1 (C), glucose transporter 2 (GLUT2; D), glucokinase (GCK; E), and glucose 6-phosphatase
614 (G6PC; F) relative to B2M. Perigonadal WAT mRNA expression changes of uncoupling protein
615 1 (UCP1; G), PGC-1α (PPARGC1A; H), INSR (I), tumor necrosis factor α (TNFA; J), and
616 interleukin 6 (IL6; K) relative to B2M. GLUT4 (L) and 1 (M) mRNA expression relative to GAPDH
617 in skeletal muscle. A two-tailed t-test was used to determine mRNA expression fold changes
618 within a genotype and sex. The number of animals is either inset or above the error bars on each
619 bar graph. *p<0.05, **p<0.01, ***p<0.001.
620
621 **Figure 5: Sexually dimorphic spatial navigation responses to thermotherapy in APP/PS1**
622 **mice.**
623 Average latency to the platform during the five MWM training sessions (A). A two-way ANOVA
624 with Sidak's post hoc was used to determine significant time differences due to thermotherapy
625 across training days. The number of platform entries (B), latency to first platform entry (C), number
626 of annulus 40 entries (D), latency to first annulus 40 entry (E), and swimming speed (F) during
627 the delayed MWM probe challenge. A two-tailed t-test was used to determine thermotherapy
628 effects on probe challenge parameters within a sex and genotype. The number of animals is inset
629 on each bar graph. *p<0.05, **p<0.01.
630
631 **Figure 6: Thermotherapy decreases hippocampal soluble Aβ_{42} in male mice.**
632 Average concentration of hippocampal soluble Aβ_{42} was determined by ELISA in male and female
633 mice. C57BL/6 mice were used as a negative control as denoted by the segmented y-axis. A two-
634 tailed t test was used to determine thermotherapy effects within a sex and genotype. The number
635 of animals is either inset or above the error bars on each bar graph. *p<0.05.


23°C Male C57BL/6
30°C Male C57BL/6
23°C Male APP/PS1
30°C Male APP/PS1
23°C Female C57BL/6
30°C Female C57BL/6
23°C Female APP/PS1
30°C Female APP/PS1
Rectal Temperature (°C)
38.0
37.5
37.0
36.5
36.0
35.0
0.0
*
**
**
18
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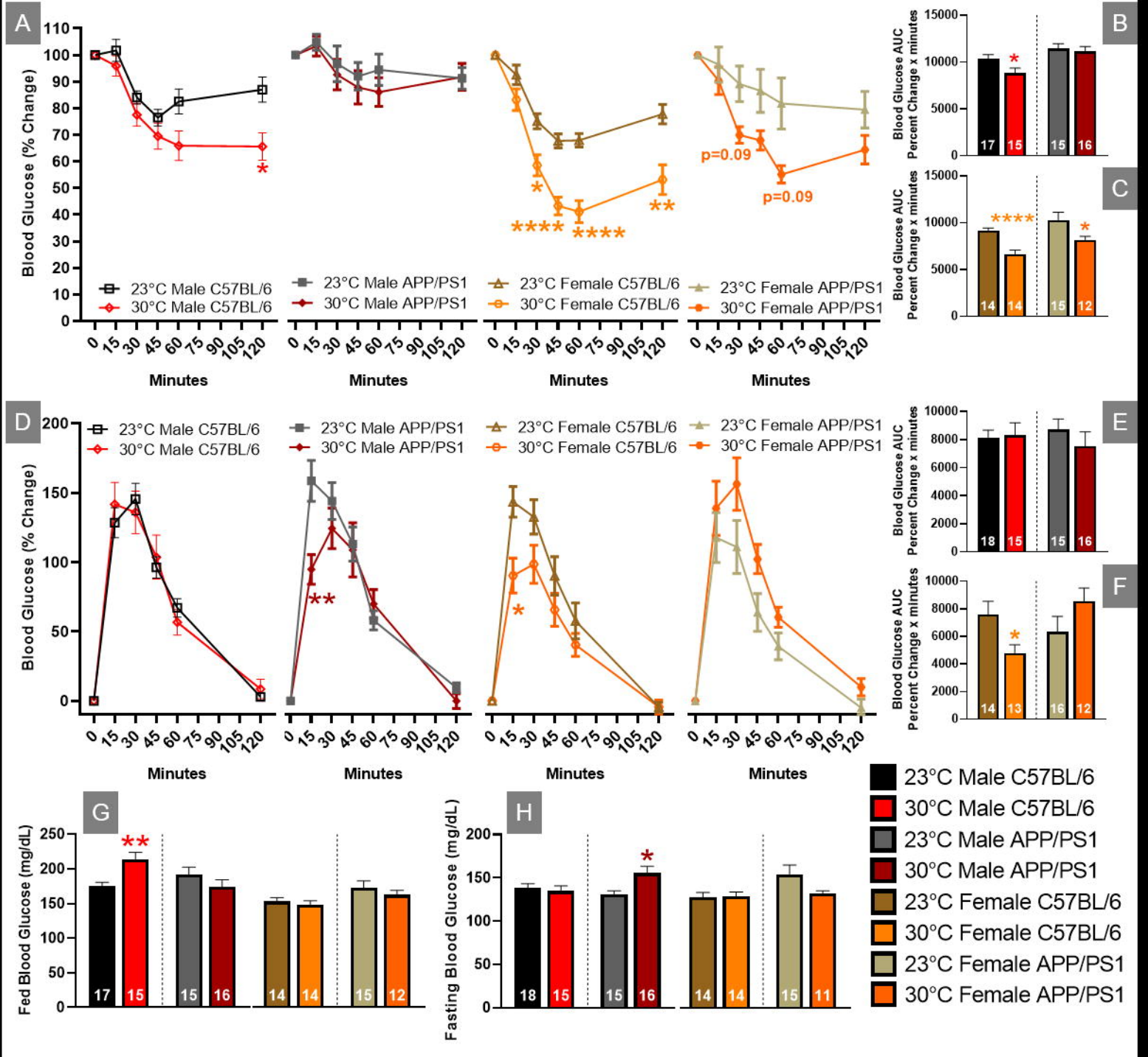
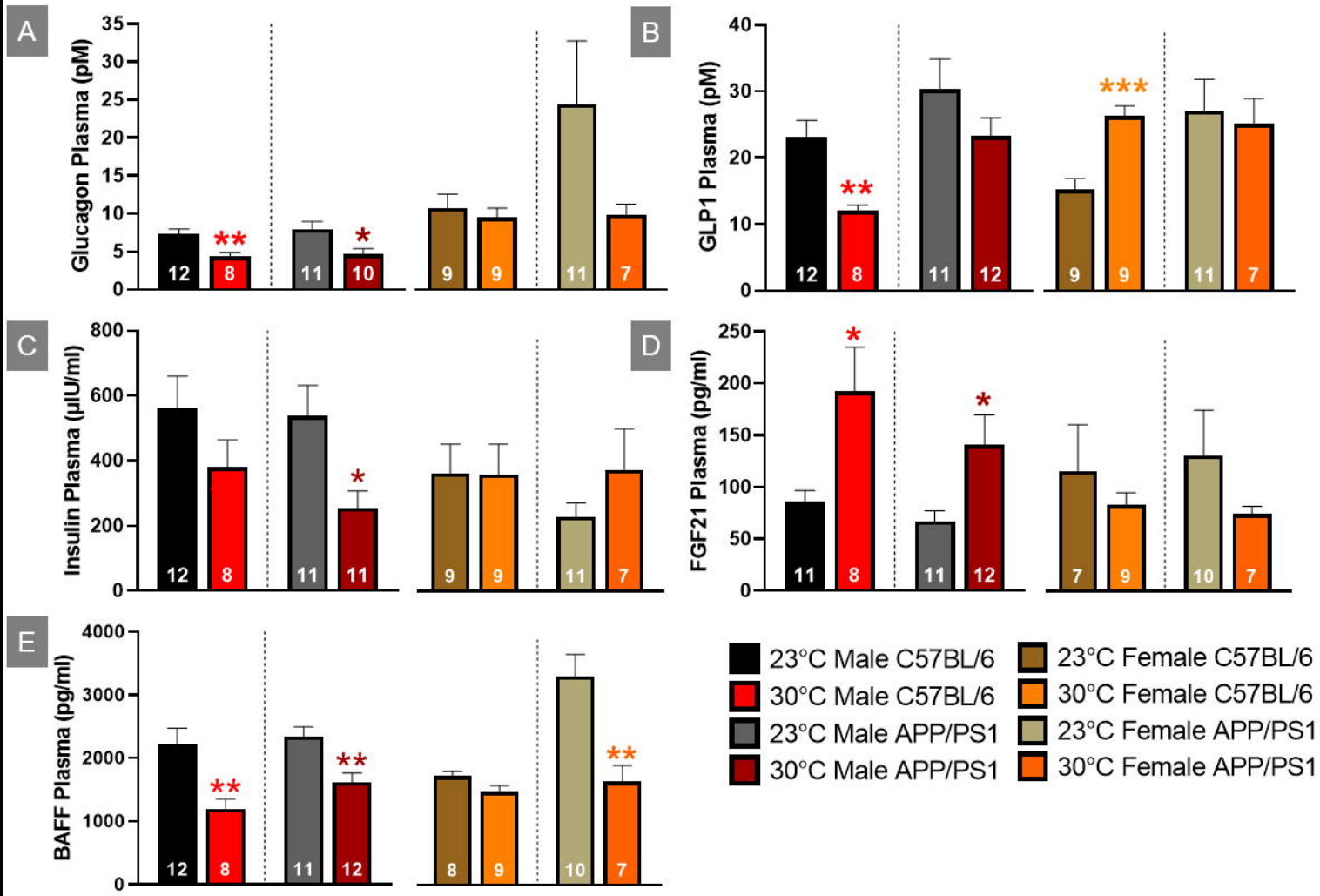

A
Blood Glucose (% Change)
Minutes
23°C Male C57BL/6
30°C Male C57BL/6
23°C Male APP/PS1
30°C Male APP/PS1
23°C Female C57BL/6
30°C Female C57BL/6
23°C Female APP/PS1
30°C Female APP/PS1
p=0.09
B
Blood Glucose AUC Percent Change x minutes
C
D
E
F
G
Fed Blood Glucose (mg/dL)
H
Fasting Blood Glucose (mg/dL)


A
Glucagon Plasma (pM)
35
30
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0
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12
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11
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11
7
B
GLP1 Plasma (pM)
40
30
20
10
0
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***
12
8
11
12
9
9
11
7
C
Insulin Plasma (µIU/ml)
800
600
400
200
0
*
12
8
11
11
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9
11
7
D
FGF21 Plasma (pg/ml)
250
200
150
100
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8
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9
10
7
E
BAFF Plasma (pg/ml)
4000
3000
2000
1000
0
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12
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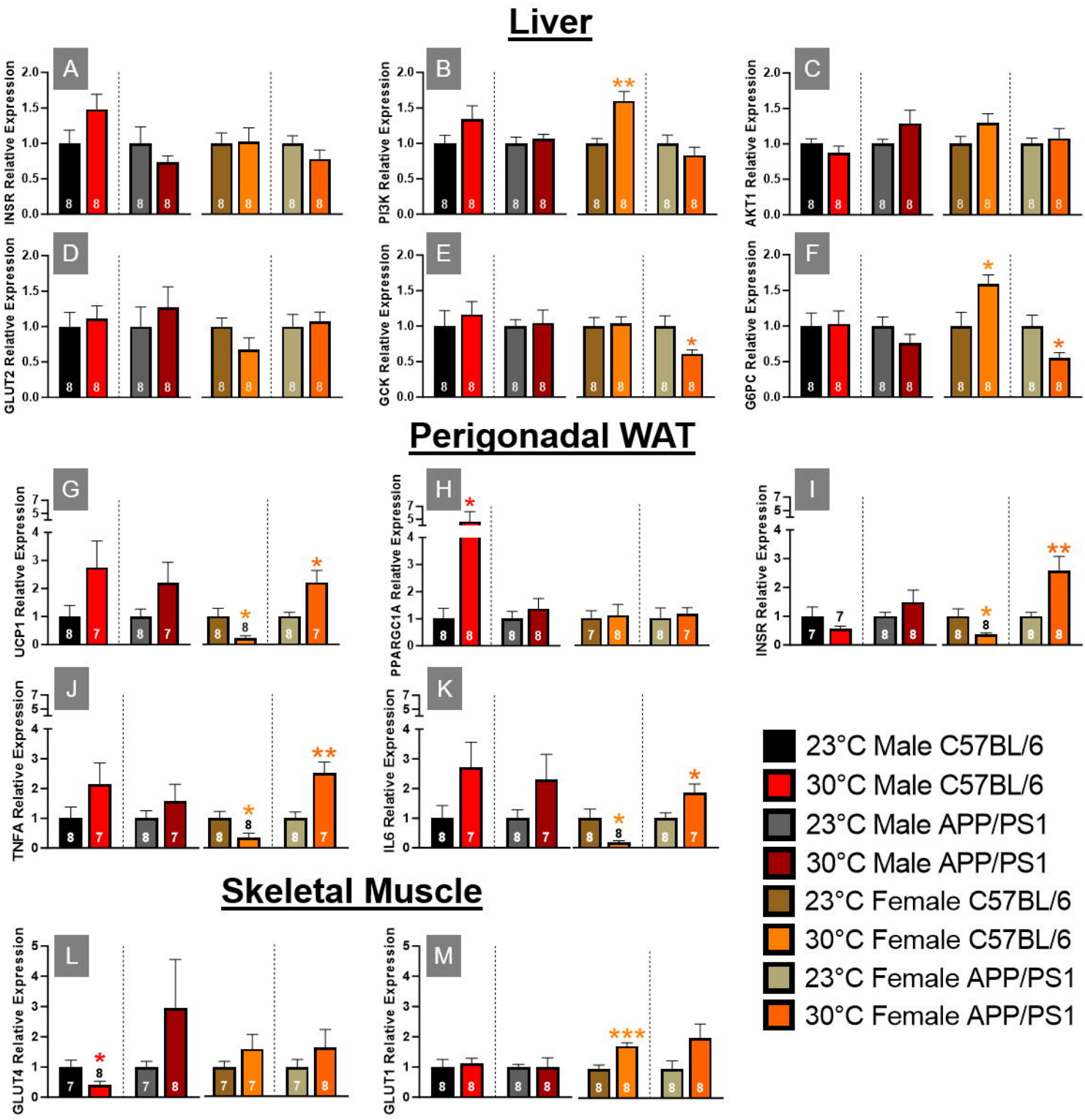
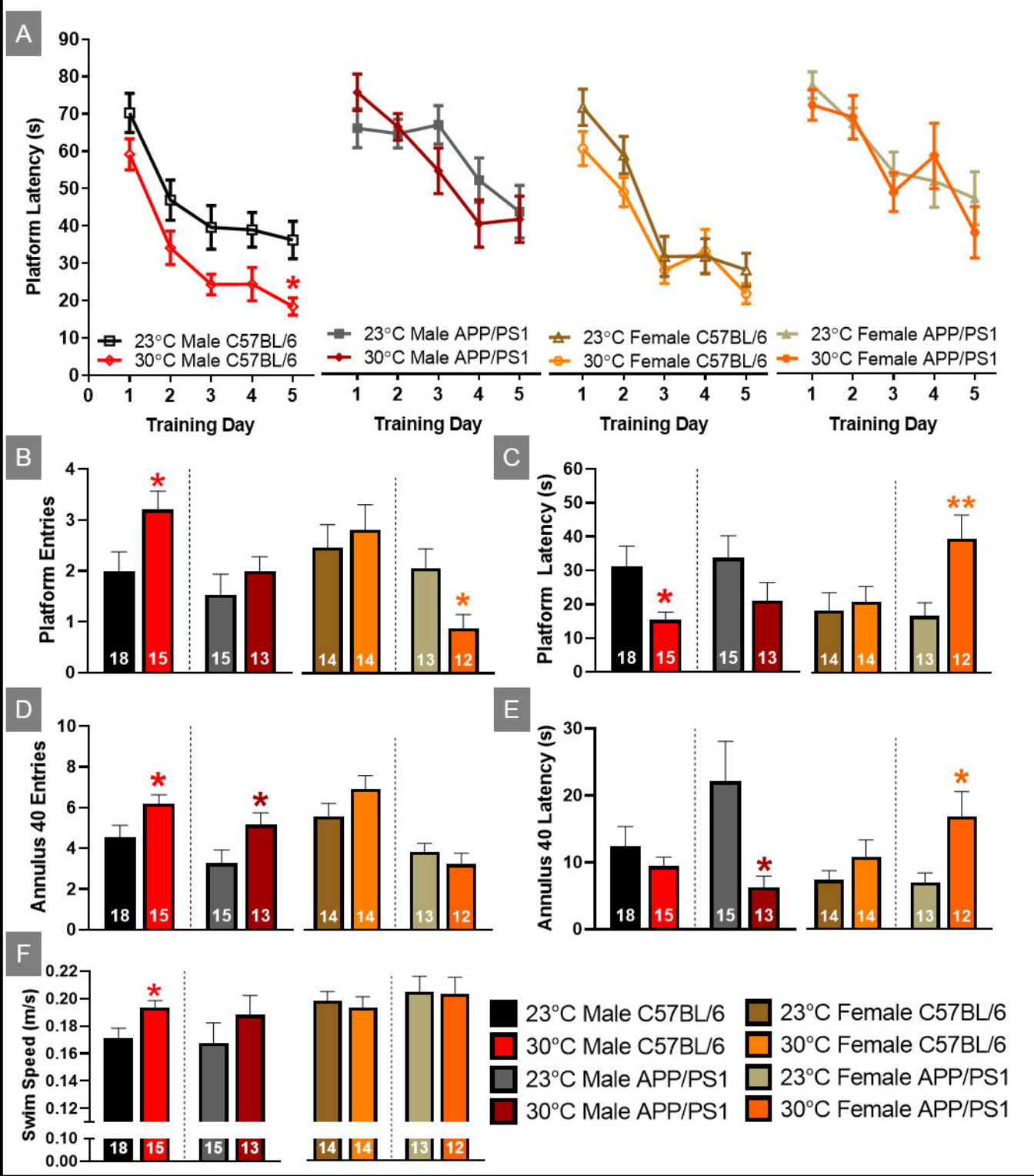
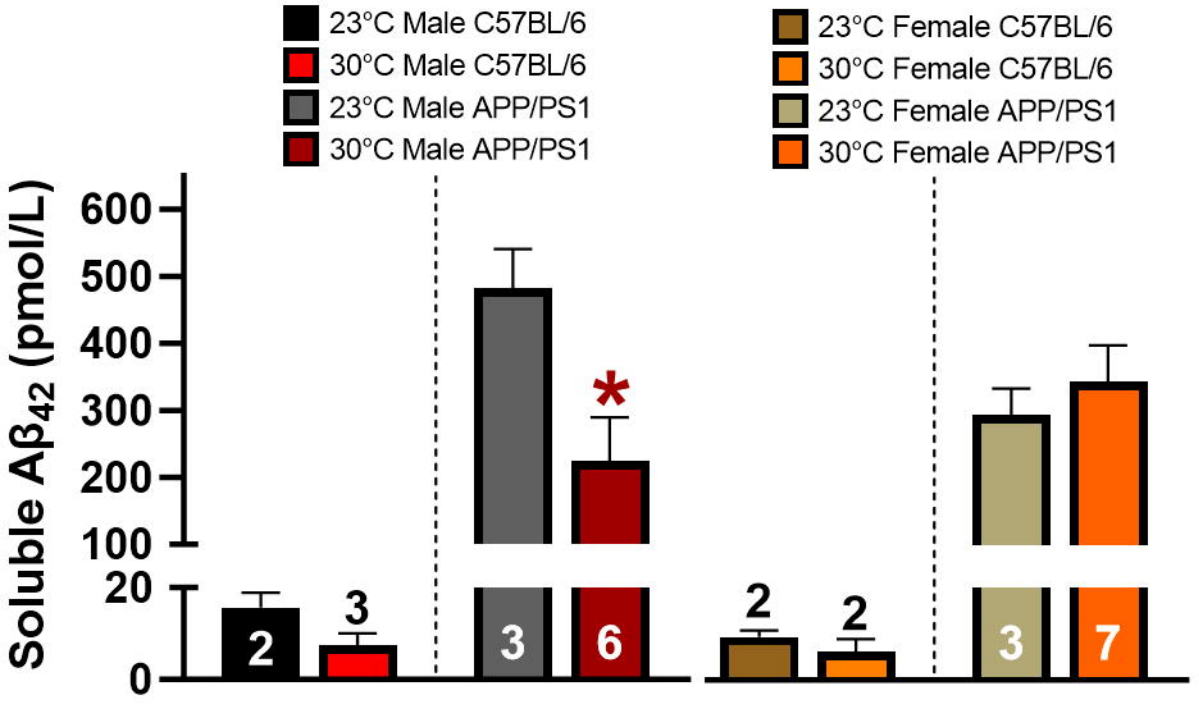
7
23°C Male C57BL/6
30°C Male C57BL/6
23°C Male APP/PS1
30°C Male APP/PS1
23°C Female C57BL/6
30°C Female C57BL/6
23°C Female APP/PS1
30°C Female APP/PS1


Liver
A
INSR Relative Expression
B
PI3K Relative Expression
C
AKT1 Relative Expression
D
GLUT2 Relative Expression
E
GCK Relative Expression
F
G6PC Relative Expression
Perigonadal WAT
G
UCP1 Relative Expression
H
PPARGC1A Relative Expression
I
INSR Relative Expression
J
TNFA Relative Expression
K
IL6 Relative Expression
Skeletal Muscle
L
GLUT4 Relative Expression
M
GLUT1 Relative Expression
23°C Male C57BL/6
30°C Male C57BL/6
23°C Male APP/PS1
30°C Male APP/PS1
23°C Female C57BL/6
30°C Female C57BL/6
23°C Female APP/PS1
30°C Female APP/PS1


A
Platform Latency (s)
Training Day
23°C Male C57BL/6
30°C Male C57BL/6
23°C Male APP/PS1
30°C Male APP/PS1
23°C Female C57BL/6
30°C Female C57BL/6
23°C Female APP/PS1
30°C Female APP/PS1
B
Platform Entries
C
Platform Latency (s)
D
Annulus 40 Entries
E
Annulus 40 Latency (s)
F
Swim Speed (m/s)


23°C Male C57BL/6
30°C Male C57BL/6
23°C Male APP/PS1
30°C Male APP/PS1
23°C Female C57BL/6
30°C Female C57BL/6
23°C Female APP/PS1
30°C Female APP/PS1
Soluble Aβ42 (pmol/L)
600
500
400
300
200
100
20
0
2
3
3
6
2
2
3
7
*