Des-acyl ghrelin prevents heatstroke-like symptoms in rats exposed to high temperature and high humidity

Yoshiyuki Inoue^a, Yujiro Hayashi^b, Kenji Kangawa^c, Yoshihiro Suzuki^d, Noboru Murakami^a, Keiko Nakahara^{a*}

^a*Department of Veterinary Physiology, Faculty of Agriculture, University of Miyazaki, Miyazaki 889-2192, Japan*

^b*Asubio Pharma Co., Ltd., 6-4-3 Minatojima-Minamimachi, Chuo-ku, Kobe 650-0047, Japan*

^c*Department of Biochemistry, National Cardiovascular Center Research Institute, 5-7-1 Fujishirodai, Suita, Osaka 565-8565, Japan*

^d*Laboratory of Animal Health Science, School of Veterinary Medicine, Kitasato University, Aomori 034-8628, Japan*

*Author for correspondence: Tel. and Fax: +81-985-58-7265

E-mail: a0d209u@cc.miyazaki-u.ac.jp (K. Nakahara)

27 **Abstract**

28 We have shown previously that des-acyl ghrelin decreases body temperature in rats
29 through activation of the parasympathetic nervous system. Here we investigated whether
30 des-acyl ghrelin ameliorates heatstroke in ratsexposed to hightemperature. Peripheral
31 administration of des-acyl ghrelin insignificantly attenuated hyperthermia induced by
32 exposure to high-temperature (35 °C)together with high humidity (70-80 %). Although
33 biochemical analysis revealed that exposure to high temperature significantly increased
34 hematocrit and the serum levels of aspartate amino transferase (AST), alanine
35 transaminase (ALT), blood urea nitrogen (BUN), creatinine and electrolytes (Na⁺, K⁺,
36 Cl⁻), most of these heatstroke-associated reactions were significantly reduced by
37 treatment with des-acyl ghrelin. The level of des-acyl ghrelin in plasma was also found to
38 be significantly increased under high-temperature conditions. These results suggest that
39 des-acyl ghrelin could be useful for preventing heatstroke under high temperature
40 condition.

41

42

43 **Abbreviations**

44 AST, aspartate amino transferase; ALT, alanine transaminase; BUN, blood urea nitrogen;
45 GOAT, ghrelin O-acyltransferase

46

47 **Keywords:**

48 Des-acyl ghrelin; Heatstroke; Body temperature

49

50

51

52

53 **1. Introduction**

54 When humans and experimental animals are exposed to a high-temperature
55 environment, heatstroke is induced. Heatstroke is characterized by hyperthermia,
56 multiple organ failure, and central nervous system disorders [1, 2]. Both disseminated
57 intravascular coagulation and inflammatory activation are believed to be responsible for
58 heatstroke-associated multiple organ dysfunction and death [3-11]. In addition, in
59 heatstroke, abnormality of electrolytes is observed, and in particular the potential
60 importance of hyperkalemia in the pathophysiology of heatstroke and heat-related
61 myocardial damage has been discussed [3-11].

62 Ghrelin was first discovered by Kojima et al. in 1999 from extracts of rat gastric tissue
63 as an endogenous ligand for GHS-R1a. Ghrelin is a 28-amino-acid peptide that is
64 characteristically octanoylated on the serine at position 3 [12]. Octanoylation of ghrelin is
65 necessary for its biological activity mediated through activation of GHS-R1a [13]. The
66 enzyme that transfers n-octanoic acid to the third serine residue of ghrelin has been
67 identified and named as ghrelin O-acyltransferase (GOAT) [14]. On the other hand,
68 des-acyl ghrelin, which lacks the serine 3 residue octanoylation, is unable to release GH
69 or bind to the classic GHS-R1a receptor [13]. These characteristics indicate that octanoic
70 acid plays an important role in physiological activity via GHS-R1a, and des-acyl ghrelin
71 has been considered an inactive form of ghrelin. However, a recent study has found that
72 des-acyl ghrelin shares some of ghrelin's effects on adiposity [15], modulation of
73 neoplastic cell proliferation and cardiovascular cell survival *in vitro* [16,17], while
74 exerting effects opposite to those of ghrelin on gastroduodenal motility [18], bone mass
75 density [19], and glucose/insulin metabolism [20, 21]. In addition, it has recently been
76 reported that des-acyl ghrelin has a variety of functions such as promotion of appetite [22],
77 inducing the production of adipose tissue, and enhancing the proliferation of human
78 osteoblastic cells through a pathway that does not involve GHS-R1a [23,24]. These

79 findings suggest the presence of an unidentified receptor specific for des-acyl ghrelin.
80 Thus, although ghrelin and des-acyl ghrelin have similar structures, their actions and the
81 mechanisms involved differ at several levels. Additionally, in our previous study, we
82 demonstrated that des-acyl ghrelin reduced body temperature by inhibiting
83 thermogenesis and facilitating heat dissipation via the parasympathetic nervous system
84 by activating the thermoregulatory center, whereas it exerted a direct action on the
85 peripheral vascular system [25].

86 Therefore, it is likely that heatstroke-induced hyperthermia and multiorgan dysfunction
87 under high-temperature conditions can be reduced by treatment with des-acyl ghrelin. In
88 order to confirm this, in the present study we investigated physiological changes in the
89 plasma levels of des-acyl ghrelin, and the effects of des-acyl ghrelin treatment on body
90 core temperature, the serum levels of organ injury indicators and electrolytes in rats
91 exposed to high-temperature and high humidity conditions.

92

93 **2. Materials and methods**

94

95 *2.1. Animals*

96

97 Male Wistar rats (Charles River Japan, Inc., Yokohama, Japan), weighing 250–300 g,
98 were housed in individual Plexiglas cages in an animal room maintained under a constant
99 light–dark cycle (lights on from 7:00 to 19:00 h), temperature (22 ± 1 °C) and humidity
100 (45–55 %). Food and water were provided ad libitum. A proportion of the rats thus housed
101 were transferred to a high-temperature (35 °C) and high-humidity(70–80 %) room. This
102 was selected as the “high-temperature and high-humidity” in this study, because a
103 preliminary experiment had shown that a lower temperature of 30 °C and lower
104 humidityof 60% was insufficient to induce heatstroke, whereas one of 38 °C exposed rats
105 to a high risk of death.All procedures were performed in accordance with the Japan
106 Physiological Society’s guidelines for animal care.

107

108 *2.2. Measurement of body temperature*

109

110 Body temperature was monitored with a rectal probe thermocouple thermometer
111 (Unique Medical Co., Ltd., Tokyo, Japan) at 10-min intervals. Rat des-acyl ghrelin (0.1
112 mg/kg body weight) or saline was administered intraperitoneally (i.p.) at 17:30, and body
113 temperature was continuously recorded every 10 min for 80 min after injection.

114

115 *2.3. Biochemical analysis of serum*

116

117 The rats were assigned randomly to one of three groups. One group (control) was placed
118 in a normal-temperature (22°C) room and given an injection of saline, whereas the

119 second and third groups were transferred to the high-temperature room (35°C) with high
120 humidity (70-80 %) at 17:00 and given injections of saline and des-acyl ghrelin (i.p.:
121 0.1 mg/kg body weight), respectively. The injections were started at 30 min after
122 introduction to the high-temperature environment, and blood samples were collected at
123 90 min after the start of high-temperature exposure. In the control group exposed to
124 normal room temperature, the schedule of saline injection or blood sampling was the
125 same as that for the experimental high-temperature exposure groups. All serum samples
126 were stored at -80 °C until measurement of the following blood parameters using an
127 automatic dry-chemistry blood analyzer (Fuji Dry-Chem. 3500 V, Fuji Medical Systems,
128 Tokyo, Japan): aspartate amino transferase (AST), alanine transaminase (ALT), blood
129 urea nitrogen (BUN), creatinine and electrolytes (Na⁺, K⁺, Cl⁻).

130

131 *2.4. Measurement of plasma des-acyl ghrelin levels*

132

133 Blood samples were collected from 8 individual rats at 0, 20, 60 and 120 min after the
134 start of exposure of high-temperature and high-humidity by the continuous tail tip
135 incision method. As a control, blood was collected continuously at the same intervals
136 from 6 individual rats housed in a standard animal room (22 ± 1 °C). Blood samples were
137 immediately placed in chilled polypropylene tubes containing 0.1 N HCl and
138 centrifuged. The level of des-acyl ghrelin in plasma was measured using specific ELISA
139 kits (Mitsubishi Kagaku Iatron, Inc., Tokyo, Japan).

140

141 *2.5. Statistical analysis*

142

143 Results are presented as mean ± S.E.M. Effects of des-acyl ghrelin on body temperature
144 were analyzed using repeated-measures ANOVA. Furthermore, differences in the

145 concentration of des-acyl ghrelin under high-temperature and high-humidity conditions
146 were analyzed by Student's *t* test. All other data were analyzed using Tukey's multiple
147 comparison tests. Values were considered to differ significantly at $p < 0.05$.

148

149 **3. Results**

150

151 *3.1. Effects of des-acyl ghrelin on body temperature in rats exposed to high temperature*

152

153 When the rats were transferred from the normal-temperature room to the
154 high-temperature and high-humidity room and administered saline 30 min later, their
155 body temperature increased gradually from about 36°C to about 40°C during a 30-min
156 period. This increase became slower and reached 41.2°C at 80 min after saline injection.
157 On the other hand, administration of des-acyl ghrelin significantly attenuated this
158 increase of body temperature, which was maintained at about 40°C for 80 min (Fig. 1).
159 The change in body temperature after saline injection differed significantly from that
160 after treatment with des-acyl-ghrelin (* $P < 0.05$).

161

162 *3.2. Effect of high temperature and des-acyl ghrelin administration on blood levels of* 163 *hematocrit, serum CRE, BUN, AST and ALT*

164

165 Rats exposed to high-temperature and high-humidity and treated with saline showed
166 significant increases in hematocrit and the serum levels of AST, ALT, BUN and
167 creatinine. On the other hand, in rats treated with des-acyl-ghrelin, there was no
168 significant increase in hematocrit or the serum levels of AST, ALT, BUN and creatinine
169 even after exposure to high-temperature and high-humidity for 90 min (Fig. 2).

170

171 3.3. *Effect of high temperature and des-acyl ghrelin administration on serum levels of*
172 *Na⁺, K⁺ and Cl⁻*

173

174 The serum levels of Na⁺, K⁺ and Cl⁻ were also significantly higher in saline-treated rats
175 exposed to hightemperature than in saline-treated rats housed at normal temperature.

176 Treatment with des-acyl ghrelin prevented the increase in the serum level of K⁺, but not
177 Na⁺and Cl⁻, in rats exposed to high-temperature and high-humidity (Fig.3).

178

179 3.4. *Plasma des-acyl ghrelin levels in rats housed under high- and normal-temperature*
180 *conditions*

181

182 To clarify the influence of high temperature on the endogenousplasma concentration
183 ofdes-acyl ghrelin, we measured the levels des-acyl ghrelin in blood after exposure to
184 high-temperature and high-humidity. A significant increase in the level of des-acyl
185 ghrelin was observed at 120 min, but not at 20 and 60 min, after the start
186 ofhigh-temperature and high-humidity exposure (Fig. 4).

187

188 **4. Discussion**

189 Heatstroke has been defined as a form of excessive hyperthermia associated with a
190 systemic inflammatory response that leads to multiple organ dysfunctions,the central
191 nervous system being predominantly affected [3]. Previous studies have shown that, in
192 comparison with normothermic controls, rats with heatstroke have a higher body core
193 temperature and higher serum and/or hypothalamic levels of toxic oxidizing radicals such
194 as nitric oxide metabolites and dihydroxybenzoic acid, systemic inflammatoryresponse
195 molecules such as tumor necrosis factor- α , interleukin-1 β and IL-6, and indicators of
196 organ injury such as creatinine, BUN, ALT and AST [26-29]. In contrast, the mean

197 arterial pressure and hypothalamic levels of cerebral blood flow and partial pressure of
198 oxygen are all significantly lower during heatstroke [27,28], and
199 electrolyte abnormalities may also be a feature.

200 In the present study also, rats exposed to high temperature together with high humidity
201 developed heatstroke-like symptoms including not only hyperthermia, an increase of
202 hematocrit, and high levels of serum creatinine, BUN, ALT and AST, but also increased
203 levels of the electrolytes Na^+ , K^+ and Cl^- . Heatstroke-induced hyperkalemia, in
204 particular, might be attributable to metabolic acidosis leading to a shift of potassium from
205 intracellular to extracellular fluid, and to liver and muscle damage causing potassium
206 release. On the other hand, treatment with des-acyl ghrelin significantly attenuated the
207 degree of hyperthermia, organ dysfunction reflected in increases of creatinine, BUN,
208 ALT and AST, and hyperkalemia in rats exposed to high temperature. We have previously
209 reported that des-acyl ghrelin decreases body temperature through stimulation of the
210 parasympathetic nervous system, since the parasympathetic nerve blocker
211 methylscopolamine, but not the sympathetic blocker timolol, inhibited the decrease of
212 body temperature induced by des-acyl ghrelin. In addition, des-acyl ghrelin more
213 potently decreases body temperature through peripheral vasodilation than ghrelin. Thus,
214 stimulation of the parasympathetic nervous system might be involved in the attenuation
215 of heatstroke-like symptoms by des-acyl ghrelin upon exposure to a high-temperature
216 environment.

217 Recently, it has been proposed that the thermoregulatory deficits induced by heatstroke
218 may result from neuronal apoptosis and cell degeneration in the hypothalamus. It has also
219 been reported that des-acyl ghrelin protects neurons from the injury induced by transient
220 focal cerebral ischemia and reperfusion in vivo, and that this effect is mediated by a
221 receptor other than GHS-R1a. Therefore, there is a possibility that in rats with heatstroke,
222 any hypothalamic apoptosis and neuronal degeneration could be significantly inhibited

223 by treatment with des-acyl ghrelin. In addition to neuronal damage in brain tissues,
224 heatstroke causes severe damage to the lung, kidney and liver [30-32]. As there is a body
225 of evidence that des-acyl ghrelin directly protects cells in several organs [17, 33, 34], the
226 present results further suggest that as well as decreasing body temperature through
227 reduction of heat production and enhancement of heat dissipation, des-acyl ghrelin also
228 a direct protective effect on various organs. This possibility will require further study.

229 The present study demonstrated that during the onset of heatstroke in rats, the plasma
230 level of des-acyl ghrelin was increased in comparison with normothermic controls. Such
231 a dramatic change in the plasma level of des-acyl ghrelin has not been reported
232 previously, and our results suggest that production of des-acyl ghrelin may be a natural
233 response designed to buffer the effects of heat stroke on the body.

234 In summary, we have demonstrated that the level of des-acyl ghrelin in plasma
235 increases significantly upon exposure to high-temperature and high-humidity
236 conditions. In addition, our biochemical assay revealed multi-organ dysfunction and
237 hyperkalemia in rats with hyperthermia that were merely treated with saline. These
238 reactions to heat stroke were all significantly reduced by treatment with des-acyl ghrelin,
239 suggesting that des-acyl ghrelin might have therapeutic benefits for patients with heat
240 stroke.

241

242

243 **Acknowledgement**

244 This study was supported in part by Grants-in-Aid from the Ministry of Education,
245 Science, Sports, and Culture of Japan, and by JST, CREST.

246

247

248 **References**

249

- 250 [1] C.C. Liu, C.H. Chien, M.T. Lin, Glucocorticoids reduce interleukin-1 concentration
251 and result in neuroprotective effects in rat heat stroke, *J. Physiol.* 27 (2000)
252 333-343
- 253 [2] A. Bouchama, J.P. Knochel, Heat stroke, *N. Engl. J. Med.* 346 (2002) 1978-1988
- 254 [3] T.C. Chao, R. Sinniah, J.E. Pakian, Acute heat stroke death, *Pathology* 13 (1981)
255 145-156
- 256 [4] A. Bouchama, R.S. Parhar, A. el-Yazigi, K. Sheth, S. al-Sedairy, Endotoxemia and
257 release of tumor necrosis factor and interleukin 1 alpha in acute heatstroke, *J. Appl.*
258 *Physiol.* 70 (1991) 2640-2644
- 259 [5] M.M. Hammami, A. Bouchama, S. al-Sedairy, E. Shail, Y. al.-Ohaly, G.E. Mohamed,
260 Concentration of soluble tumor necrosis factor and interleukin-6 receptors in heat
261 stroke and heat stress, *Crit. Care Med.* 25 (1997) 1314-1319
- 262 [6] A. Bouchama, M.M. Hammami, E. Al Shail, E. De Vol, Differential effects of in vitro
263 and in vivo hyperthermia on the production of interleukin-10, *Intensive Care Med.*
264 26 (2000) 1646-1651
- 265 [7] M.T. Lin, T.Y. Kao, C.F. Su, Hsu. SS, Interleukin-1 beta production during the onset
266 of heat stroke in rabbits, *Neurosci. Lett* 174 (1994) 17-20
- 267 [8] M.T. Lin, H.H. Liu, Y.L. Yang, Involvement of interleukin-1 receptor mechanisms in
268 development of arterial hypotension in rat heat stroke, *Am. J. Physiol*, 273 (1997)
269 2072-2077
- 270 [9] A. Bouchama, G. Roberts, F. el-Mohanna, R el-Sayed, B. Lach, S .Chollet-Martin, V
271 Ollivier, R al-Baradei, A. Loualich, S. Nakeeb, A. Eldali, D. De Prost,
272 Inflammatory, hemostatic and clinical changes in a baboon experimental model for
273 heat stroke, *J. Appl. Physiol.* 98 (2005) 697-705
- 274 [10] C.K. Chang, C.P. Chang, W.T. Chiu M.T. Lin, Prevention and repair of circulatory
275 shock and cerebral ischemia/injury by various agents in experimental heatstroke,
276 *Curr. Med. Chem.* 13 (2006) 3145-3154
- 277 [11] K.H. Shen, C.H. Lin, H.K. Chang, W.C. Chen, S.H. Chen, Premarin can act via
278 estrogen receptors to rescue mice from heatstroke-induced lethality, *Shock* 30
279 (2008) 668-674
- 280 [12] M. Kojima, H. Hosoda, Y. Date, M. Nakazato, H. Matsuo, K. Kangawa, Ghrelin is a
281 growth-hormone-releasing acylated peptide from stomach, *Nature* 402 (1999)

282 656-660

283 [13] H. Hosoda, M. Kojima, H. Matsuo, K. Kangawa, Ghrelin and des-acyl ghrelin: two
 284 major forms of rat ghrelin peptide in gastrointestinal tissue, *Biochem. Biophys. Res.*
 285 *Commun.* 279 (2000) 909-913

286 [14] H. Ohgusu, K. Shirouzu, Y. Nakamura, Y. Nakashima, T. Ida, T Sato, M. Kojima,
 287 Ghrelin O-acyltransferase (goat) has a preference for N-hexanoyl-coa over
 288 N-octanoyl-coa as an acyl donor, *Biochem. Biophys. Res. Commun.* 386 (2009)
 289 153-158

290 [15] W. Zhang, B. Chai, J.Y. Li, H. Wang, M.W. Mulholl, Effect of des-acyl ghrelin on
 291 adiposity and glucose metabolism, *Endocrinology* 149 (2008) 4710-4716

292 [16] P. Cassoni, C. Ghé, T. Marrocco, E. Tarabra, E. Allia, F. Catapano, R. Deghenghi, E.
 293 Ghigo, M. Papotti, G. Muccioli, Expression of ghrelin and biological activity of
 294 specific receptors for ghrelin and des-acyl ghrelin in human prostate neoplasms and
 295 related cell lines, *Eur. J. Endocrinol.* 150 (2004) 173-84

296 [17] G. Baldanzi, N. Filigheddu, S. Cutrupi, F. Catapano, S. Bonisconi, A. Fubini, D.
 297 Malan, G. Baj, R. Granata, F. Broglio, M. Papotti, N. Surico, F. Bussolino, J.
 298 Isgaard, R. Deghenghi, F. Sinigaglia, M. Prat, G. Muccioli, E. Ghigo, A. Graziani,
 299 Ghrelin and des-acylghrelininhibitcelldeath in cardiomyocytes and endothelialcells
 300 through ERK1/2 and PI3-kinase/AKT, *J. Cell. Biol.* 159 (2002) 1029-1037

301 [18] M. Fujimiya, A. Asakawa, K. Ataka, I. Kato, A. Inui, Different effects of ghrelin,
 302 des-acyl ghrelin and obestatin on gastroduodenal motility in conscious rats, *World J.*
 303 *Gastroenterol.* 14 (2008) 6318-6326

304 [19] L. Pacifico, C. Anania, E. Poggiogalle, J.F. Osborn, G. Prossomariti, F. Martino, C.
 305 Chiesa, Relationships of acylated and des-acyl ghrelin levels to bone mineralization
 306 in obese children and adolescents, *Bone* 45 (2009) 274-279

307 [20] R.M. Kiewiet, M.O. van Aken, K. van der Weerd, P. Uitterlinden, A.P. Themmen,
 308 L.J. Hofland, Y.B. de Rijke, P.J. Delhanty, E. Ghigo, T. Abribat, A.J. van der Lely,
 309 Effects of acute administration of acylated and unacylated ghrelin on glucose and
 310 insulin concentrations in morbidly obese subjects without overt diabetes, *Eur. J.*
 311 *Endocrinol.* 161 (2009) 567-573

312 [21] C. Gauna, F.M. Meyler, J.A. Janssen, P.J. Delhanty, T. Abribat, P. van Koetsveld, L.J.
 313 Hofland, F. Broglio, E. Ghigo, A.J. van der Lely, Administration of acylated ghrelin
 314 reduces insulin sensitivity, whereas the combination of acylated plus unacylated
 315 ghrelin strongly improves insulin sensitivity, *J. Clin. Endocrinol.Metab.* 89 (2004)

316 5035-5042

317 [22] K. Toshinai, H. Yamaguchi, Y. Sun, R.G. Smith, A. Yamanaka, T. Sakurai, Y. Date,
318 M.S. Mondal, T. Shimbara, T. Kawagoe, N. Murakami, M. Miyazato, K. Kangawa,
319 M. Nakazato, Des-acyl ghrelin induces food intake by a mechanism independent of
320 the growth hormone secretagogue receptor, *Endocrinology* 147 (2006) 2306-2314

321 [23] N.M. Thompson, D.A. Gill, R. Davies, N. Loveridge, P.A. Houston, I.C. Robinson, T.
322 Wells, Ghrelin and des-octanoyl ghrelin promote adipogenesis directly in vivo by a
323 mechanism independent of the type 1a growth hormone secretagogue receptor,
324 *Endocrinology* 145 (2004) 234-242

325 [24] P.J. Delhanty, B.C. van der Eerden, M. van der Velde, C. Gauna, H.A. Pols, H. Jahr,
326 H. Chiba, A.J. van der Lely, J.P. van Leeuwen, Ghrelin and unacylated ghrelin
327 stimulate human osteoblast growth via mitogen-activated protein kinase
328 (MAPK)/phosphoinositide 3-kinase (PI3K) pathways in the absence of GHS-R1a, *J.*
329 *Endocrinol* 188 (2006) 37-47

330 [25] Y. Inoue, K. Nakahara, K. Maruyama, Y. Suzuki, Y. Hayashi, K. Kangawa, N.
331 Murakami, Central and peripheral des-acyl ghrelin regulates body temperature in
332 rats, *Biochem. Biophys. Res. Commun.* 430 (2013) 278-283

333 [26] W.S. Liu, C.T. Chen, N.H. Foo, H.R. Huang, J.J. Wang, S.H. Chen, T.J. Chen,
334 Human umbilical cord blood cells protect against hypothalamic apoptosis and
335 systemic inflammation response during heatstroke in rats, *Pediatr. Neonatol.* 50
336 (2009) 208-216

337 [27] S.H. Hsiao, C.P. Chang, T.H. Chiu, M.T. Lin, Resuscitation from experimental
338 heatstroke by brain cooling therapy, *Resuscitation* 73 (2007) 437-445

339 [28] X.J. Lin, Y.L. Li, G.P. Mei, F. Zou, D.D. He, X.Q. Liu, Y.J. Li, T.B. Zhao, M.T. Lin,
340 Activated protein C can be used as a prophylactic as well as a therapeutic agent for
341 heat stroke in rodents, *Shock* 32 (2009) 524-529

342 [29] H.H. Yang, C.P. Chang, J.T. Cheng M.T. Lin, Inhibition of acute lung inflammation
343 and injury is a target of brain cooling after heatstroke injury, *J. Trauma.* 69 (2010)
344 805-812

345 [30] C.K. Chang, C.P. Chang, W.T. Chiu, M.T. Lin, Prevention and repair of circulatory
346 shock and cerebral ischemia/injury by various agents in experimental heatstroke,
347 *Curr. Med. Chem.* 13 (2006) 3145-3154

348 [31] W.S. Liu, C.T. Chen, N.H. Foo, H.R. Huang, J.J. Wang, S.H. Chen, T.J. Chen,
349 Human umbilical cord blood cells protect against hypothalamic apoptosis and

350 systemic inflammation response during heatstroke in rats, *Pediatr. Neonatol.* 50
351 (2009) 208-216

352 [32] H.H. Yang, C.P. Chang, J.T. Cheng, M.T. Lin, Inhibition of acute lung inflammation
353 and injury is a target of brain cooling after heatstroke injury, *J. Trauma.* 69 (2010)
354 805-812

355 [33] R. Granata, F. Settanni, L. Biancone, L. Trovato, R. Nano, F. Bertuzzi, S. Destefanis,
356 M. Annunziata, M. Martinetti, F. Catapano, C. Ghè, J. Isgaard, M. Papotti, E. Ghigo,
357 G. Muccioli, Acylated and unacylated ghrelin promote proliferation and inhibit
358 apoptosis of pancreatic β -cells and human islets: involvement of 3',5'-cyclic
359 adenosine monophosphate/protein kinase A, extracellular signal-regulated kinase
360 1/2, and phosphatidyl inositol 3-Kinase/Akt signaling, *Endocrinology* 148 (2007)
361 512-529

362 [34] L. Li, L.K. Zhang, Y.Z. Pang, C.S. Pan, Y.F. Qi, L. Chen, X. Wang, C.S. Tang, J.
363 Zhang, Cardioprotective effects of ghrelin and des-octanoyl ghrelin on myocardial
364 injury induced by isoproterenol in rats, *ActaPharmacol. Sin.* 27 (2006) 527-35
365
366

367 **Figure legends**

368

369 Figure 1.

370 The effects of saline (n=12) and des-acyl ghrelin (n=10) on body temperature in rats
371 exposed to high-temperature (35°C) and high-humidity (70-80 %). Rat des-acyl ghrelin or
372 saline was administered i.p. at 17:30 and body temperature was continuously recorded
373 every 10 minutes for 80 minutes after injection. Arrow indicates the day of administration
374 of des-acyl ghrelin and saline. Each symbol and vertical line represents the mean $\pm$ SEM.
375 There were significant differences between the groups (*P < 0.05).

376

377 Figure 2.

378 Effect of high-temperature and des-acyl ghrelin administration on blood levels of
379 hematocrit, serum CRE, BUN, AST and ALT in rats. Clear bar represents the mean level
380 ($\pm$ SEM, n=8) in rats placed in a normal-temperature room (22°C) and treated with
381 saline. Gray and black bars represent the mean levels ($\pm$ SEM, each n=8) in rats placed in
382 a high-temperature (35°C) and high-humidity (70-80 %) room and treated with saline
383 (gray bar) or des-acyl ghrelin (black bar). Parameters were determined in rats at 90 min
384 after initiation of heat exposure. There were significant differences between the groups
385 (*P < 0.05).

386

387 Figure 3.

388 Effect of high temperature and des-acyl ghrelin administration on serum levels of Na⁺,
389 K⁺ and Cl⁻ in rats. Clear bar represents the mean level ($\pm$ SEM, n=8) in rats placed in a
390 normal-temperature room (22°C) and treated with saline. Gray and black bars represent
391 the mean levels ($\pm$ SEM, each n=8) in rats placed in a high-temperature (35°C) and
392 high-humidity (70-80 %) room and treated with saline (gray bar) or des-acyl ghrelin

393 (black bar). Parameters were determined in rats at 90 min after initiation of heat exposure.

394 There were significant differences between the groups (*P <0.05).

395

396 Figure 4.

397 Plasma des-acyl ghrelin levels in rats housed under high- and normal-temperature

398 conditions. Six control rats placed in a normal-temperature room (22°C: clear bar) and 8

399 rats placed in a high-temperature (35°C) and high-humidity (70-80 %) room (black

400 bar) were subjected to continuous collection of blood samples by the tail tip incision

401 method at 20, 60, and 120 min after the start of exposure to high temperature. Zero min

402 means just before the start of exposure to high temperature. Each bar represents the mean

403 $\pm$ SEM. Significant differences relative to the saline group (*P <0.05) are indicated.

404

405

406

Fig. 1

Fig. 2

Fig. 3

Fig.4