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The role of the tryptophan-nicotinamide pathway in a model of severe malnutrition induced liver dysfunction

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32 **Abstract**

33 Mortality in children with severe malnutrition is strongly related to signs of metabolic dysfunction,
34 such as hypoglycemia. Lower circulating tryptophan levels in children with severe malnutrition
35 suggest a possible disturbance in the tryptophan-nicotinamide (TRP-NAM) pathway and
36 subsequently NAD⁺ dependent metabolism regulator sirtuin1 (SIRT1). We report that severe
37 malnutrition in weanling mice, induced by feeding a low protein diet, leads to an impaired TRP-
38 NAM pathway and affects hepatic mitochondrial turnover and function. We demonstrate that
39 stimulating the TRP-NAM pathway improves hepatic mitochondrial and overall metabolic
40 function which is dependent on SIRT1. Activating SIRT1 is sufficient to induce improvement in
41 metabolic functions. Our findings indicate that modulating the TRP-NAM pathway can partially
42 improve liver metabolic function in severe malnutrition and could lead to the development of new
43 interventions for children with severe malnutrition.

44 **Introduction**

45 Malnutrition contributes to nearly 45% of deaths among children under 5 years of age worldwide¹.
46 Malnourished children, especially those with severe malnutrition are at a substantially increased
47 risk of mortality compared to well-nourished children². The current treatment guidelines
48 developed by the World Health Organization (WHO) for children with severe malnutrition are
49 based on limited scientific evidence³. Thus, new evidence-based interventions are urgently needed.

50 The liver is a central organ that regulates nutrient metabolism. In severe malnutrition, hepatic
51 metabolism has been found to be disturbed and is associated with hypoglycemia,
52 hypoalbuminemia, and steatosis⁴⁻⁶. Children with severe malnutrition have impaired hepatic
53 glucose production, which increases the risk of hypoglycemia and is related to mortality⁵. We
54 recently discovered in both patients and a rodent model of severe malnutrition, that hepatic
55 mitochondrial function is impaired leading to reduced nutrient oxidation and adenosine
56 triphosphate (ATP) depletion^{5,6}. However, the pathophysiology of hepatic mitochondrial
57 dysfunction in severe malnutrition remains poorly understood.

58 Children with severe malnutrition have been found to have significantly lower serum tryptophan
59 levels⁷⁻⁹. As an essential amino acid, tryptophan is crucial for growth and protein synthesis. It is
60 also a precursor of nicotinamide adenine dinucleotide (NAD⁺) and nicotinamide adenine
61 dinucleotide phosphate (NADP⁺), which are essential co-factors in metabolic and biosynthesis
62 pathways. We have previously shown that higher excretion of *N*-methylnicotinamide, a urinary
63 biomarker of NAD⁺ and nicotinamide availability, was associated with catch-up growth in stunted
64 infants¹⁰. NAD⁺ is also a co-substrate for sirtuin1 (SIRT1), which is an important enzyme for
65 mitochondrial health and biogenesis through activation of peroxisome proliferator-activated
66 receptor-gamma coactivator-1 alpha (PGC-1 α)¹¹. SIRT1 has also been shown to regulate
67 autophagy¹²⁻¹⁴. There have been reports that targeting this pathway in non-alcoholic fatty liver
68 disease (NAFLD) has beneficial effects on hepatic metabolism¹⁵⁻¹⁸. The role of tryptophan
69 nicotinamide (TRP-NAM) pathway in severe malnutrition-associated hepatic metabolic
70 dysfunction remains unknown.

71 In this study we aimed to characterize the role of the TRP-NAM pathway in hepatic metabolic
72 dysfunction in a mouse model of severe malnutrition. We demonstrate that the TRP-NAM pathway
73 is affected in this model and that hepatic mitochondrial dysfunction is related to deficiencies in the
74 TRP-NAM pathway. We demonstrate supplementing with NAM and related components of this
75 pathway improve mitochondrial and overall hepatic metabolic dysfunction. We find that the effects
76 of modulating the TRP-NAM pathway are mediated through SIRT1. These findings identify the
77 importance of the TRP-NAM pathway and SIRT1 in malnutrition-associated hepatic metabolic
78 dysfunction.

79

80 **Results**

81 **Feeding a low protein diet leads to hepatic steatosis in young mice.**

82 To develop a mouse model of severe malnutrition, we fed 3-weeks-old weanling male C57BL/6J
83 mice a 1% protein isocaloric diet for two weeks (malnourished group) and compared it to the
84 control group fed an 18% protein diet (control group) (Fig. 1a). Mice subjected to the 1% protein
85 diet lost a significant amount of body weight (approximately 20%) over two weeks and had a lower
86 body length and weight for length ratio compared to the 18% protein-fed control group (Fig. 1b-
87 d). The 1% protein-fed mice showed a lower liver weight and liver to body weight ratio compared
88 to control (Fig. 1e). Lower glucose concentrations were also noted in the 1% protein-fed mice
89 before and after fasting (Fig. 1f), consistent with reduced hepatic glucose production. The
90 respiratory exchange ratio (RER) was lower during the dark phase and higher during the light
91 phase in 1% protein-fed mice, indicating a loss of the day-night feeding cycle in this group. Energy
92 expenditure was lower in 1% protein-fed mice compared to the 18% protein-fed control group
93 (Fig. 1g).

94 Histological H&E staining and Oil Red O staining of the livers identified steatosis in the mice fed
95 with 1% protein diet as evidenced by an increase in fat vacuoles and larger fat droplets compared
96 to the mice fed with 18% protein diet (Fig 2a-b). The increase in lipid droplets in the liver of the
97 1% protein-fed mice was confirmed by immunofluorescence staining with BODIPY (Fig. 2c).
98 Further quantification of histology slides showed consistency with these observations (Fig. 2d)

99 and was validated by measurement of liver triglyceride (TG) levels (Fig. 2e). Serum TGs were
100 lower in the 1% protein-fed group, indicating steatosis is not linked to hypertriglyceridemia (Fig.
101 2f). Together, these results indicate that the 1% protein diet induces hepatic steatosis in mice
102 similar to those observed in patients and rat model of severe malnutrition^{2,6}.

103

104 **NAM and TRP-NAM pathway modulators reduce the development of low protein diet-** 105 **induced hepatic steatosis.**

106 Examination for blood tryptophan levels showed the 1% protein diet mice to be lower than 18%
107 protein diet control animals ($43.0 \pm 5.0 \mu\text{mol/L}$ and $88.4 \pm 13.2 \mu\text{mol/L}$ respectively, $p=0.0035$). To
108 examine the role of a reduced tryptophan levels and possible nicotinamide (NAM) deficiency on
109 liver health, the 1% protein-fed mice were supplemented with 160 mg/kg body weight NAM from
110 day 7 to day 14 (Fig. 1a). NAM treatment did not alter the average body weight, body length, or
111 food and liquid consumption in the 1% protein-fed group (Fig. 1b-d). The mice treated with NAM
112 had no significant difference in liver weight, liver/body weight ratio, or fasting glucose levels
113 compared to the untreated 1% protein diet-fed mice (Fig. 1e-f). RER and energy expenditure were
114 not affected by NAM treatment (Fig. 1g). NAM treatment improved the hepatic steatosis compared
115 to the 1% protein-fed mice, indicated by a reduction in the fat vacuoles area and a 30% reduction
116 in liver TG levels compared to untreated animals (Fig. 2a-e). The NAM treatment had no effect on
117 serum TG concentrations (Fig. 2f).

118 To determine whether the effect of NAM treatment was due to improvement of the NAD salvage
119 pathway specifically, we treated the 1% protein-fed mice with nicotinamide riboside (NR) or
120 tryptophan (TRP). Both NR and TRP act as NAD⁺ precursors in the NAD salvage pathway¹⁶. The
121 allocated interventions were given from day 7 to day 14 (Supplementary Fig. 1a). NR and TRP
122 supplementation, similar to NAM treatment, did not recover body weight, body length or liver
123 weight/body weight ratio compared to the untreated 1% protein-fed group (Supplementary Fig.
124 1b-e). Similar to the NAM treated malnourished mice, hepatic steatosis was reduced in the NR and
125 TRP treated groups (Supplementary Fig. 2a-f). To determine whether the effects were specific to
126 the TRP-NAM pathway, we also performed similar experiments in mice who received
127 supplementation with methionine (MET), another essential amino acid like tryptophan. This

128 particular amino acid was chosen as MET has been shown to decrease hepatic steatosis in mice on
129 ketogenic diets¹⁹, and diets completely devoid of MET and choline can induce hepatic
130 steatosis^{20,21}. Supplementation with methionine did not improve hepatic steatosis among the 1%
131 protein-fed mice (Supplementary Fig. 2a-f). MET supplementation also did not recover body
132 weight and body length, but increased liver weight and body weight ratio in comparison to the
133 untreated 1% protein-fed mice alone (Supplementary Fig. 1b-d). Together, these results indicate
134 that supplementation of different NAD⁺ precursors improve low protein-induced hepatic steatosis.

135

136 **NAM improves low protein diet-induced mitochondrial changes.**

137 To further understand the mechanisms underlying the improved hepatic steatosis in response to
138 NAM treatment, we next evaluated changes in hepatic mitochondrial characteristics in our model.
139 We have previously shown that protein-deficient diet induces mitochondrial morphological and
140 functional changes and reduces mitochondrial activity in rats under protein restricted diet⁶. In our
141 mouse model, immunofluorescent staining of mitochondria in the liver showed that the
142 mitochondria were enlarged and elongated but decreased in numbers in the 1% protein-fed mice
143 compared to the 18% protein-fed control group (Fig. 3a). The loss of mitochondria was further
144 confirmed by a significant decrease in the mitochondrial DNA (mtDNA) copy number (Fig. 3b).
145 This feature improved after NAM, NR, and TRP treatment (Fig. 3b, Supplementary Fig. 2h).
146 Mitochondrial abundance markers including TOM20 and HSP60 were both significantly lower in
147 the 1% protein diet-fed mice compared to the control, but improved with NAM treatment (Fig.
148 3c,d). This suggests that NAM treatment can either reduce mitochondria degradation or increase
149 its biogenesis in our model of severe malnutrition.

150 To examine mitochondrial fitness, we examined hepatic ATP levels, and levels of mitochondrial
151 complex proteins. Further, we quantified the expression of genes in the β -oxidation and lipogenesis
152 pathway. The livers of the 1% protein-fed malnourished mice had significantly lower hepatic ATP
153 levels compared to the 18% protein-fed control group (Fig. 3e). NAM and other TRP-NAM
154 pathway modulators significantly restored hepatic ATP levels (Fig. 3e, Supplementary Fig. 2i).
155 Complex I, Complex IV, and Complex V protein levels were significantly lower in the 1% protein-
156 fed group compared to the control group (Fig. 3f,g). Complex IV levels improved significantly

157 after NAM treatment, while no significant change was observed in levels of other complexes.
158 Expression of the genes in the β -oxidation pathway was reduced in the livers of mice fed a 1%
159 protein diet and were partially restored after NAM treatment, especially Acaa2 and Hadha (Fig.
160 3h). The expression of lipogenesis genes including Fasn and Acaca were decreased in mice fed a
161 1% protein diet (Fig. 3i). NAM supplementation did not influence the mRNA expression of
162 lipogenesis genes (Fig. 3i). In summary, feeding mice a 1% protein diet altered the hepatic
163 mitochondrial morphology, decreased mitochondrial number and mass, and affected markers of
164 oxidative phosphorylation and β -oxidation. NAM treatment improved the 1% protein diet-induced
165 mitochondrial changes associated with a recovery in ATP content.

166

167 **A low protein diet leads to changes in hepatic energy metabolism that improve with NAM**
168 **treatment.**

169 To better understand the overall liver metabolic change in mice fed with 1% protein diet and
170 evaluate the effect of NAM supplementation, we performed quantitative analysis of liver central
171 carbon metabolism metabolites²². The major metabolic profile differences between groups was
172 highlighted by sparse-partial least squares-discriminant analysis (sPLS-DA)²³. Variable
173 importance in projection (VIP) scores were used to identify the most important metabolites for the
174 clustering. Overall, the hepatic metabolic profiles of the 1% protein diet-fed malnourished group
175 were clearly separated from those of the 18% protein diet-fed control group, and distinct from
176 NAM treatment group (Fig. 4a-b). Among the metabolomic features, acetylglucosamine-1P,
177 glyceraldehyde-3P, malonyl-CoA, lactic acid, ATP, erythrose-4P, UMP, UDP-glucose, glucose,
178 pyruvic acid, and ADP-glucose mostly discriminated 18% protein diet from 1% protein diet
179 groups, with variable importance in projection (VIP) score >1 in both components 1 and 2 (Fig.
180 4a). To be more specific, the 1% protein-fed group showed significantly lower glucose, lactic acid,
181 and pyruvic acid content compared to control (Supplementary Table 1)²⁴. GMP and UMP
182 concentrations decreased in the 1% protein diet-fed group, suggesting disturbed nucleotide
183 metabolism including pyrimidine and purine synthesis. Malonyl-CoA levels also changed in the
184 1% protein-diet fed group, consistent with altered lipogenesis^{25,26}. The overall results were also in
185 line with an earlier report of impaired ATP production and decreased pyruvate uptake,

186 accompanied by altered tricarboxylic acid cycle (TCA) cycle intermediates in a rat model of
187 malnutrition⁶. Modulation of the TRP-NAM pathway altered hepatic metabolic profiles as
188 observed by sPLS-DA (Fig. 4b and Supplementary Fig. 3a). NAM treatment shifted malonyl-CoA,
189 UTP, ATP, Hs-CoA, UDP-Glucose, total fructose-bisP/glucose-1,6-bisP, acetyl-CoA, AMP, and
190 succinyl-CoA, which mostly differentiate them with 1% protein diet group (VIP score >1). The
191 concentration of ATP, malonyl-CoA, and acetyl-CoA in NAM treated group shifted towards the
192 18% protein diet-fed control group, which was related to the improved energy production and
193 carbohydrate and lipid metabolism (Supplementary Table 1)²⁷.

194 To further explore the changes in lipid metabolism in our model and evaluate the effect of TRP-
195 NAM modulation, we performed a lipidomic analyses. Overall, discriminating features were
196 identified that clearly separate the 18% protein diet and 1% protein diet group, dominated by
197 increased levels of triacylglycerols, diacylglycerols, and sterols (VIP score >1) (Fig. 4c and
198 Supplementary Table 2). Interestingly, hepatic phospholipid content was lower in the 1% group
199 compared to the 18% group. The decreased PC/TG ratio and phosphatidylcholines to
200 phosphatidylethanolamines ratio (PC/PE) in the 1% protein diet group might be linked to the
201 altered energy metabolism and lipid droplet size and dynamics^{28,29}. Decreased PC/PE ratios have
202 also been observed in NASH patients^{30,31}, potentially through mitochondrial respiratory chain
203 dysfunction and disability to meet energy requirements³². NAM treatment clearly separated this
204 group from the 1% protein diet group and separation was primarily caused differences in
205 phosphatidylcholines and diacylglycerols (VIP score >1) (Fig. 4d and Supplementary Table 2).
206 NR and TRP treatment groups were close to each other but clearly separate from MET treatment
207 group, mostly highlighted by altered triacylglycerols and diacylglycerols (with VIP score >1)
208 (Supplementary Fig. 3b).

209

210 **NAM treatment affects NAD⁺ and the SIRT1 pathway in low protein-fed mice.**

211 To determine whether NAM treatment directly affects the NAD salvage pathway, we measured
212 the abundance of hepatic NAD⁺ and tryptophan pathway metabolites in the liver of these animals.
213 NAD⁺ levels and many metabolites in the tryptophan pathway (such as kynurenine, kynurenine
214 acid, serotonin) were decreased in the 1% protein-fed mice compared to the 18% protein-fed

215 control group (Fig. 5a). NAM treatment increased hepatic nicotinic acid concentrations, indicating
216 NAM was bioavailable and affected the TRP-NAM pathway. However, we did not observe a
217 significant effect of NAM treatment on NAD⁺ levels itself ($p = 0.640$), whereas NR treatment did
218 significantly increase hepatic NAD⁺ levels (Supplementary Fig. 2j). This result is consistent with
219 other studies that have reported that NR increased hepatic NAD⁺ levels³³. Another chronic NAM
220 supplementation study showed that NAM did not boost NAD⁺ but enhanced the de-acetylation of
221 SIRT1 targets¹⁸.

222 Next, we investigated changes in the NAD dependent SIRT1 pathway. The protein levels of SIRT1
223 and its downstream target PGC-1 α were significantly decreased in the mice fed a 1% protein diet
224 compared to the 18% protein-fed control group and levels of these proteins were significantly
225 improved after NAM treatment, albeit not to the same level as the control group (Fig. 5b,e). The
226 ratio of p65 to Ac-p65 significantly increased in the 1% protein-fed group compared to the control,
227 which was improved after NAM treatment, indicating a change in SIRT1 deacetylation activity
228 (Fig. 5c,e).

229 Since SIRT1 has been shown to influence autophagy and we previously showed an impairment in
230 autophagy flux in livers of low protein-fed rodents⁶, we next evaluated autophagy levels by
231 measuring microtubule-associated protein 1A/1B-light chain 3 (LC3) LC3-I and LC3-II protein
232 levels. Autophagy pathway marker of LC3-II/LC3-I ratio significantly decreased in the 1%
233 protein-fed malnourished group compared to the 18% protein-fed control group, suggesting a
234 decrease in autophagy activation (Fig. 5d,e). NAM treatment increased the LC3-II/LC3-I ratio,
235 which suggests an increase in activation of macro-autophagy. Taken together, our results suggest
236 that the TRP-NAM pathway is disturbed after feeding a 1% protein diet to mice and that it can be
237 partially restored by NAM treatment. In turn, the improvement in the TRP-NAM pathway elevates
238 SIRT1 which may be linked to the increase in PGC-1 α and activation of autophagy.

239

240 **The effect of NAM on low protein diet-induced liver metabolic dysfunction is mediated**
241 **through SIRT1.**

242 To further test the whether the effect of NAM is SIRT1-dependent, we performed experiments
243 using SIRT1 modulators in the 1% protein-fed mice with or without NAM supplementation (Fig.
244 6a). The SIRT1 activator, resveratrol (REV)^{34,35}, was used to investigate if SIRT1 activation was
245 sufficient to demonstrate an improvement in the hepatic metabolic changes caused by 1% protein
246 feeding. The SIRT1 inhibitor, selisistat (EX-527)^{36,37}, was subsequently used in combination with
247 NAM treatment to determine if the effect of NAM was dependent on the activation of SIRT1.

248 Intraperitoneal injection of REV did not change body weight, body length and liver weight
249 compared to the vehicle control group (Fig. 6b-e). However, we observed a decrease in the degree
250 of hepatic steatosis in the 1% protein-fed malnourished group treated with REV, with nearly 2
251 folds decrease in fat vacuole area and decreased liver TG levels compared to untreated 1% protein
252 fed animals (Fig. 7a,b). mtDNA copy number and ATP levels significantly increased after REV
253 treatment (Fig. 7c,d). Among the β -oxidation genes, we observed small but significant increases
254 in Hadha and Acadm expression after REV treatment, without a significant change in expression
255 of lipogenesis genes compared to vehicle treated group (Fig. 7e,f). When the 1% protein-fed
256 malnourished mice were treated with both EX-527 and NAM, the effects of NAM treatment on
257 hepatic steatosis and mtDNA copy number were lost (Fig. 7a-c). SIRT1 protein level was
258 upregulated after REV treatment (Fig. 7g). There was also a trend toward increased PGC-1 α
259 protein levels in the REV treated group (p-value = 0.083). EX-527 with NAM treatment also did
260 not affect SIRT1 and PGC-1 α levels compared to the 1% protein-fed malnourished group alone
261 (Fig. 7g). These data indicate that the SIRT1 increase is sufficient to improve 1% protein diet-
262 induced hepatic metabolic dysfunction and the effect of NAM treatment on hepatic metabolism is
263 dependent on the elevation of SIRT1.

264

265 **Discussion**

266 Our study indicates that feeding weanling mice a 1% protein diet leads to stunted growth, severe
267 wasting, hepatic lipid accumulation and mitochondrial dysfunction that is associated with a
268 reduction in activity in SIRT1, PGC-1 α and autophagy. We demonstrate that supplementing the
269 TRP-NAM pathway is able to improve the metabolic phenotype and that this effect is dependent

270 on SIRT1. This is the first report on the role of the TRP-NAM pathway in a murine malnutrition
271 model.

272 The hepatic metabolic changes induced by the protein deficient diet were consistent with our
273 previous findings in a rat model of severe malnutrition showing liver steatosis and ATP depletion
274 caused by mitochondrial dysfunction in a rat model of severe malnutrition¹¹. The data are also
275 consistent with limited reports in children with severe malnutrition that have found impaired
276 mitochondrial function^{4,5}. Interestingly, there is considerable overlap with features seen in patients
277 with NAFLD, including changes in mitochondrial complexes, mitochondrial biogenesis, and
278 hepatic lipid accumulation³⁸⁻⁴⁰. The reduction in mitochondrial mass seen in our mouse model is
279 different from previous observations in low protein fed rats, where an increase in mitochondrial
280 mass was observed⁶. However, reduction in mtDNA in our low protein diet mouse model was
281 consistent with another previous report in fetal and early postnatal malnourished rats fed a low
282 casein diet⁴¹.

283 The reduction in mitochondrial mass and mtDNA in low protein-fed mice was associated with a
284 reduction in PGC-1 α , a well-known regulator of cellular energy metabolism and activator of
285 mitochondrial biogenesis^{42,43}. PGC-1 α can co-activate transcription factors such as peroxisome
286 proliferator-activated receptor (PPAR α) and nuclear respiratory factors (NRF1 and NRF2) to
287 regulate mitochondrial biogenesis and fatty acid oxidation⁴⁴. Mice that are deficient in PGC-1 α
288 have impaired energy metabolism that is related to a decrease in mitochondrial number and
289 respiratory capacity⁴⁵. This suggests that the reduction in mitochondrial mass is related to a
290 decrease in mitochondrial biogenesis upon low protein feeding. The changes in mitochondrial
291 morphology, mitochondrial complex content, and markers of mitochondrial function, such as ATP,
292 also indicate that the mitochondria that are present in the liver after a period of low protein feeding
293 are damaged and dysfunctional. Mitochondrial degradation is regulated through a selective
294 autophagy process called mitophagy¹², and our data suggests that autophagy activation is
295 decreased during nutritional stress. This could contribute to a high relative content of damaged
296 mitochondria that would normally have been degraded through mitophagy. NAM treatment
297 increased PGC-1 α protein levels, mitochondrial mass and content of mitochondrial complexes,
298 while activating the autophagy pathway, suggesting a rebalancing of mitochondrial biogenesis and
299 mitophagy.

300 PGC-1 α and autophagy are both regulated by SIRT1. SIRT1 directly deacetylates PGC-1 α at
301 multiple lysine sites and the induction pattern of SIRT1 protein correlates with the expression of
302 PGC-1 α ⁴⁶. In addition, SIRT1 regulate autophagy by acting on multiple autophagy effectors. These
303 mechanisms include directly inducing autophagy by deacetylating autophagy-related genes (ATGs)
304 and LC3, indirectly inhibiting the mTOR pathway by activation of AMPK, as well as modulating
305 the expression of autophagy and mitophagy regulatory molecules (e.g. Rab7 and Bnip3) through
306 deacetylation of Forkhead box O transcription factors (FOXOs)^{47,48}. SIRT1 levels were decreased
307 in our low protein diet-fed mice. As SIRT1 activity is dependent on NAD availability, we propose
308 that lower SIRT1 activity is associated with reduced levels of NAD and other metabolites in the
309 TRP-NAM pathway in low protein diet-fed mice. Supplementing these protein deficient animals
310 with NAM was found to rescue SIRT1 mediated activity. We propose that the reduction in NAD
311 prevents the SIRT1 mediated activation of PGC-1 α and autophagy pathway. Our results are
312 consistent with a clinical study reporting that increased malnutrition risk was associated with
313 decreased SIRT1 expression⁴⁹. The decreased protein levels of SIRT1 found after low protein
314 feeding could potentially be explained by diet-triggered cleavage of SIRT1 protein. For example,
315 a high-fat diet has been shown to induce SIRT1 protein cleavage leading to metabolic
316 dysfunction⁵⁰.

317 NAM was shown to increase SIRT1 levels. The effect was not specific to NAM, as NR and TRP
318 demonstrated a similar effect. Other NAD⁺ precursors such as NR and TRP have demonstrated a
319 similar effect in previous studies^{17,51,52}. We focused on NAM specifically for more in depth
320 investigations because of its low cost and excellent safety profile. Treatment with NAM and other
321 NAD⁺ precursors have shown beneficial effects in various metabolic dysfunction models,
322 including fatty liver, obesity, metabolic syndrome, and diabetes^{18,53,54}. The beneficial effects in
323 these studies have been related to an improved mitochondrial function, mediated by NAD⁺
324 dependent sirtuin activation^{17,51,52}. Our SIRT1 modulation experiments demonstrated that in our
325 malnutrition model the effects of NAM were dependent on the presence of SIRT1 and that
326 stimulating SIRT1 was sufficient to produce the beneficial effects on mitochondrial function. The
327 results are consistent with studies in high fat-fed mice where resveratrol impacted mitochondrial
328 function and prevented hepatic steatosis³⁴.

329 In our study, NAM treatment did not significantly restore NAD⁺ levels whereas NR did, however
330 NAM improved SIRT1 and PGC-1 α levels. Some studies have shown that NAM has the ability to
331 increase cellular and blood NAD⁺ content in different metabolic disorder models (*e.g.* NAFLD
332 mice, hepatocytes with endoplasmic reticulum stress)⁵⁵⁻⁵⁸. However, other studies have found no
333 direct effect of NAM supplementation on NAD⁺ levels^{18,59}. If the extra NAD that is synthesized,
334 is readily used for deacetylation, then you would not see a significant increase. These differences
335 in findings might also be related to the duration and variation in the dose of NAM and the animal
336 models used affecting NAM metabolism. For example, NAM can affect SIRT1 activity differently
337 by acting as a non-competitive end-product inhibitor and as a NAD⁺ precursor⁶⁰. In addition,
338 NAM clearance pathways through MNAM-mediated SIRT1 protein stabilization can also regulate
339 hepatic nutrient metabolism^{61,62}.

340 In conclusion, this work provides evidence for the role of TRP-NAM pathway in liver metabolic
341 dysfunction in a mouse model of severe malnutrition, mediated through changes in levels of SIRT1.
342 This study improves our understanding of the cellular pathophysiology of severe malnutrition. The
343 results of this project could lead to the development of new interventions that target the TRP-NAM
344 pathway which could then be taken to clinical trials.

345

346 **Methods**

347 **Animals and diets.** A breeding colony of C57BL/6 mice was obtained from Jackson Laboratories
348 (Bar Harbor, ME, USA). Male mice at 3 weeks post-partum were weaned and housed socially in
349 filtered cages at The Hospital for Sick Children, Toronto. Weanling male C57BL/6J mice were
350 randomized into different groups fed with control diet (18% protein) or malnourished diet (1%
351 protein) for a period of 2 weeks. Diets were purchased from ENVIGO (Madison, WI, USA), and
352 the protein proportions contribute to diet calories were primarily adjusted by casein and corn
353 starch. After 7 days, malnourished subgroups were treated with modulators of the TRP-NAM
354 pathway until sacrifice on day 14. Nicotinamide, nicotinamide-riboside and tryptophan were given
355 by drinking water in a dose of 160 mg/kg body weight/day, and methionine was included in diets
356 at a concentration of 0.75 g/kg diet^{15,59,63}. Nicotinamide, nicotinamide-riboside and tryptophan

357 were provided by Sigma-Aldrich (St. Louis, MO, USA). In a subset of mice, after 1% protein diet
358 for 7 days, intraperitoneal injections treated with either resveratrol (25 mg/kg/d) or EX-527
359 (10 mg/kg/d) with NAM were given for 7 consecutive days until sacrifice^{36,37,64}. All groups were
360 housed in a temperature-controlled environment (23 °C), 12 h light-dark cycle, and had ad libitum
361 access to diet and water throughout the study. All animal experiments were approved by the
362 Animal Care Committee of The Hospital for Sick Children, Toronto (Animal Use Protocol
363 Number: 1000030900).

364 **Physiological parameters.** Body weight, food intake, and liquid intake were monitored from day
365 1 to day 14. At the end of the experimental protocol (on day 14 post weaning), mice were humanely
366 euthanized and necropsied. Final body weight, body length, and liver weight were recorded. Blood
367 was collected by cardiac puncture. Liver tissue was collected for histology or stored at -80 °C for
368 later use in biochemical analyses. Glucose concentration was determined via tail snip at 0h, 4h,
369 8h, and 12h fasting in the day light cycle, using an automatic glucometer (Freestyle, Abbott, IL).
370 Metabolic rate was assessed by indirect calorimetry using the Columbus Instruments (Oxymax
371 Lab Animal Monitoring System: CLAMS, Columbus, OH)¹⁸.

372 **Histology.** Fresh livers tissues were fixed in 4% paraformaldehyde (PFA) overnight at 4 °C and
373 then embedded in either paraffin or optimum cutting temperature (OCT) compound. Liver paraffin
374 sections (5 µm) were stained with hematoxylin and eosin (H&E) for morphology. Liver OCT
375 sections were stained with Oil red O (10 µm) for lipid droplets. Slides were visualized under a
376 light microscope and was measured using Panoramic Viewer version 1.15 software (3DHISTECH
377 Ltd, Budapest, Hungary). For each slide, at least five pictures were captured. Quantification
378 analysis of the images was conducted using ImageJ 1.52v and Python 3.7.2.

379 **Immunofluorescence.** OCT-embedded liver sections were cut into 4 µm slices for
380 immunofluorescent staining. A fluorinated boron-dipyrromethene (BODYPY) antibody was used
381 to visualize fat droplets. An HSP60 antibody was used to visualize mitochondrial morphology.
382 Nuclei were counterstained with DAPI. Slides were mounted with mounting medium (Vector
383 Laboratories Inc., Burlington, Canada) and images were acquired on a Nikon Spinning Disk
384 Confocal Microscope (Nikon Inc., NY, USA). Additional information can be found in the
385 Supplementary Table 3.

386 **Plasma tryptophan analysis.** Plasma samples were mixed with equal volumes of internal standard
387 (Norleucine). Samples were centrifuged at 14000 rpm for 5 minutes and subsequently measured
388 on Biochrom 30+ Amino Acid Analyzer (Biochrom, Cambridge, UK).

389 **Triglyceride analysis.** Liver and serum TG concentrations were quantified by a commercially
390 available kit (Randox, London, UK). Liver tissue lipids were extracted with methanol-chloroform,
391 dried and dissolved for TG analysis. Values were also normalized to protein concentrations
392 determined using a bicinchoninic acid assay (BCA) kit (Thermo Fisher Scientific, USA).

393 **Western blotting.** Western blot analysis was conducted to measure the protein levels. Liver tissue
394 protein was extracted through sonication of tissue with extraction buffer and protease inhibitor
395 cocktail (Sigma-Aldrich). The protein concentration was measured using pierce BCA kit (Thermo
396 Fisher Scientific). Equal concentrations of the samples were electrophoresed through 4%-12% Bis
397 Tris gel and transferred onto a polyvinylidene fluoride (PVDF) membrane. Membranes were
398 probed with 1:1000 dilutions of anti HSP60 (Abcam, USA), TOM20 (Santa Cruz, USA), Complex
399 I (Abcam, USA), Complex IV (Abcam, USA), Complex V (Abcam, USA), SIRT1 (Cell
400 Signalling, USA), PGC-1 α (Abcam, USA), p65 (Abcam, USA), Ac-p65 (Abcam, USA), LC3B
401 (Sigma, USA). β -actin (Sigma, USA) was used as a loading control in 1:1000 dilution. Then
402 proteins were visualized using a pierce enhanced chemiluminescence (ECL) plus kit (Invitrogen,
403 CA, USA). Western blot quantification was performed using Image Studio (LI-COR Biosciences).
404 Additional information can be found in the Supplementary Table 3.

405 **qPCR.** Total RNA was isolated from frozen liver tissue using Direct-zol RNA MiniPrep Kit
406 (ZYMO research Inc., Irvine, CA, USA). cDNA was synthesized by the Super Script VILO cDNA
407 Synthesis Kit (Thermo Fisher Scientific, USA). 500 ng of liver total RNA were used for cDNA
408 synthesis. Ribosomal protein l13a (Rpl13a) was used as reference gene. qPCR was performed on
409 CFX384 Touch Real-Time PCR Detection System (Bio-Rad, CA, USA). For mtDNA copy
410 number measurements, 500 ng of genomic DNA were used for each qPCR reaction and β -globin
411 were used as reference⁶⁵. Additional information can be found in the Supplementary Table 4.

412 **Metabolomic analysis.** Targeted metabolomic profiling (pathway specific assays) was performed
413 by The Metabolomics Innovation Centre (TMIC, Edmonton, AB Canada). The quantitation of
414 central carbon metabolism metabolites in mouse liver was measured by ultraperformance liquid

415 chromatography-tandem mass spectrometry (UPLC-MS/MS). A Dionex 3400 UHPLC system
416 coupled to a 4000 QTRAP mass spectrometer was used. The MS instrument was operated in the
417 multiple-reaction monitoring (MRM) mode with negative-ion (-) or positive-ion (+) detection,
418 depending on which groups of metabolites were measured. Each liver tissue sample was frozen
419 and placed into an Eppendorf tube. Water, at 2 μL per mg tissue, was added and the samples were
420 homogenized for 1 min twice at a shaking frequency of 30 Hz, with the aid of two 4-mm metal
421 balls, on a MM 400 mill mixer. After a short-time centrifuge, methanol, at 8 μL per mg tissue, was
422 added and the samples were homogenized again for 1 min twice using the same settings. The
423 samples were then sonicated in an ice-water bath for 3 min, followed by centrifugal clarification
424 at 15,000 rpm and 5 $^{\circ}\text{C}$ in an Eppendorf 5424R centrifuge for 20 min. The clear supernatants were
425 collected to conduct quantitation of TCA cycle carboxylic acids, glucose and selected sugar
426 phosphates, and other phosphate-containing metabolites and nucleotides by UPLC-MS/MS.
427 Concentrations of the detected metabolites were calculated from their linear-regression calibration
428 curves with internal calibration. Tryptophan pathway metabolites were also measured using a
429 UPLC-MS based targeted method⁶⁶.

430 **Lipidomic analysis.** Lipidomic analysis was performed at Core Metabolomics and Lipidomics
431 Laboratory, Wellcome Trust-Metabolic Research Laboratories (University of Cambridge,
432 Cambridge, UK). Liver samples were homogenised, lipids were extracted according to a published
433 procedure, and data was acquired through Direct Infusion Mass Spectrometry (DI-MS)⁶⁷. Briefly,
434 liver sections (30 mg/each) were homogenised (Tissue homogeniser II, Qiagen) in a buffer of
435 chaotropes (guanidinium chloride (6 M) and thiourea (1.5 M) in deionised water, 500 μL /sample).
436 The liver homogenates (30 μL) were injected into a well (96w plate, Esslab Plate+TM, 2.4 mL/well,
437 glass-coated) followed by methanol spiked with internal standards (150 μL), water (500 μL) and
438 DMT (500 μL , dichloromethane, methanol and triethylammonium chloride, 3:1:0.005). Most of
439 the aqueous solution was removed (96 channel pipette). A portion of the organic solution (20 μL)
440 was transferred to a high throughput plate (384 w, glass coated, Esslab Plate+TM) before being
441 dried (N_2 (g)). The dried films were re-dissolved (TBME, 30 μL /well) and diluted with a stock
442 mixture of alcohols and ammonium acetate (100 μL /well; propan-2-ol: methanol, 2:1;
443 $\text{CH}_3\text{COONH}_4$ 7.5 mM). The analytical plate was heat-sealed and run immediately. Lipid fraction
444 isolates were profiled using a three-part method in DI-MS⁶⁷. All samples were infused into an
445 Exactive Orbitrap (Thermo, Hemel Hempstead, UK), using a TriVersa NanoMate (Advion, Ithaca

446 US). Samples (15 μ L) were sprayed at 1.2 kV in the positive ion mode. The Exactive started
447 acquiring data 20 s after sample aspiration began. The Exactive acquired data with a scan rate of
448 1 Hz (resulting in a mass resolution of 100,000 full width at half-maximum [fwhm] at 400 m/z).
449 The Automatic Gain Control was set to 3,000,000 and the maximum ion injection time to 50 ms.
450 After 72 s of acquisition in positive mode the NanoMate and the Exactive switched over to
451 negative ionization mode, decreasing the voltage to -1.5 kV and the maximum ion injection time
452 to 50 ms. The spray was maintained for another 66 s, after which the NanoMate and Exactive
453 switched over to negative mode with in-source fragmentation (also known as collision-induced
454 dissociation, CID; 70 eV) for a further 66 s. After this time, the spray was stopped and the tip
455 discarded, before the analysis of the next sample began. The sample plate was kept at 15 $^{\circ}$ C
456 throughout the acquisition. Samples were run in row order. The instrument was operated in full
457 scan mode from m/z 150-1200 Da (for singly charged species).

458 **Statistical analysis.** Statistical significance for the difference between two groups was calculated
459 by using an unpaired two-tailed student's T-test. Statistical significance for the difference among
460 more than two groups was calculated by using an ordinary one-way ANOVA followed by the
461 Turkey's post hoc test⁶⁸. The FDR and Bonferroni correction were applied to the metabolomic and
462 lipidomic metabolites. Statistical analysis was performed with R software (version 3.5.2) and
463 MetaboAnalyst (version 4.0). Statistical significance was given as * $p < 0.05$, ** $p < 0.01$, and
464 *** $p < 0.001$. The results are expressed as mean \pm standard error of mean (S.E.M.), unless
465 otherwise indicated.

466

467 **Data availability**

468 All relevant data of this study are available within the paper and its supplementary information
469 files. All data that support this study are available from the corresponding authors upon reasonable
470 request.

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634

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640

641 **Author contributions**

642 R.H.J.B. and G.H. were primarily responsible for the study design. G.H. wrote the manuscript.
643 G.H., C.L., L.C., S.F., J.S., M.K.T., D.L., M.C., C.J.V., and G.B.G. contributed to the conduction
644 of lab experiments. G.H., L.C., S.F., and J.S. contributed to data analysis. P.K.K., A.K., and B.M.B.
645 provided expertise, interpreted results, and commented on the manuscript. All authors contributed
646 to editing of the manuscript. R.H.J.B. was responsible for the final content of the manuscript.

647

648 **Competing interests**

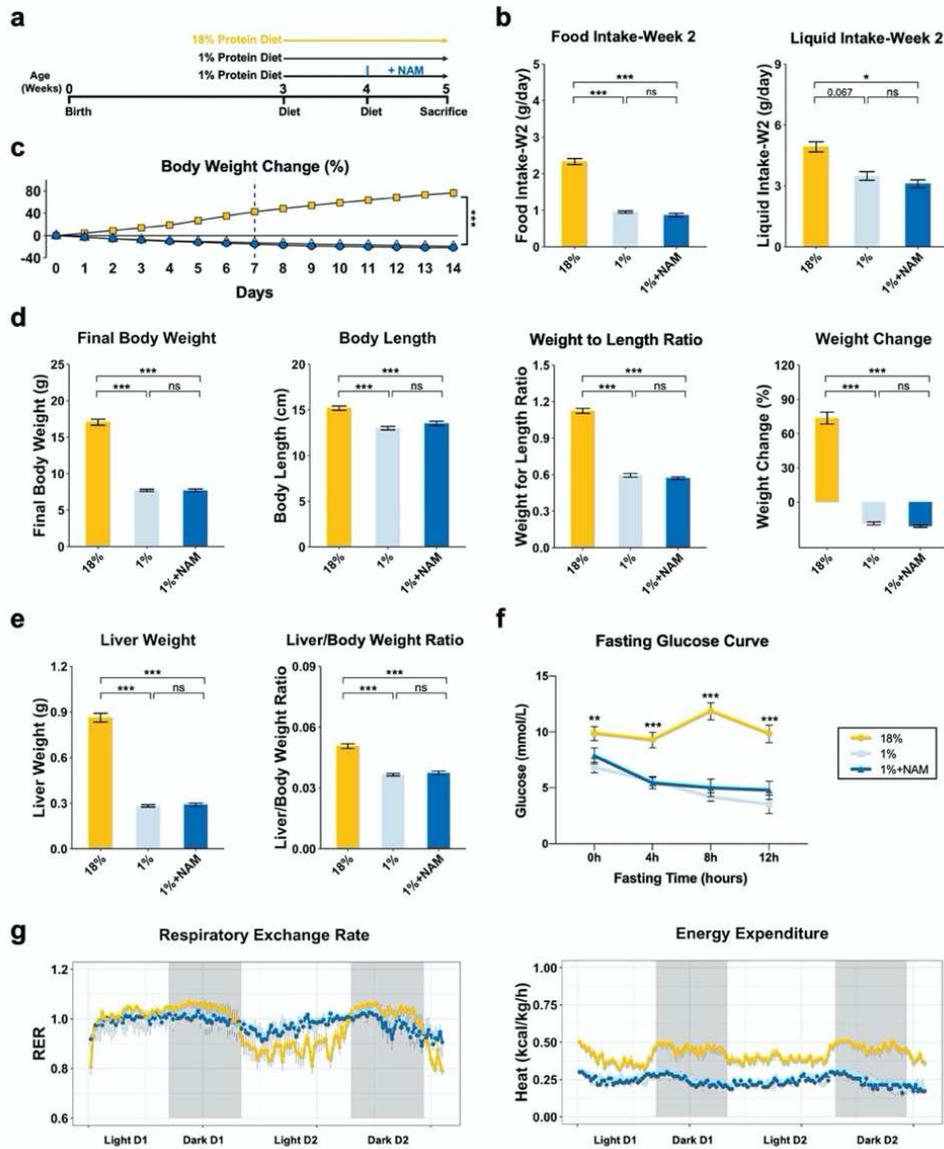
649 All participants declare no competing interests.

650

651 **Additional information**

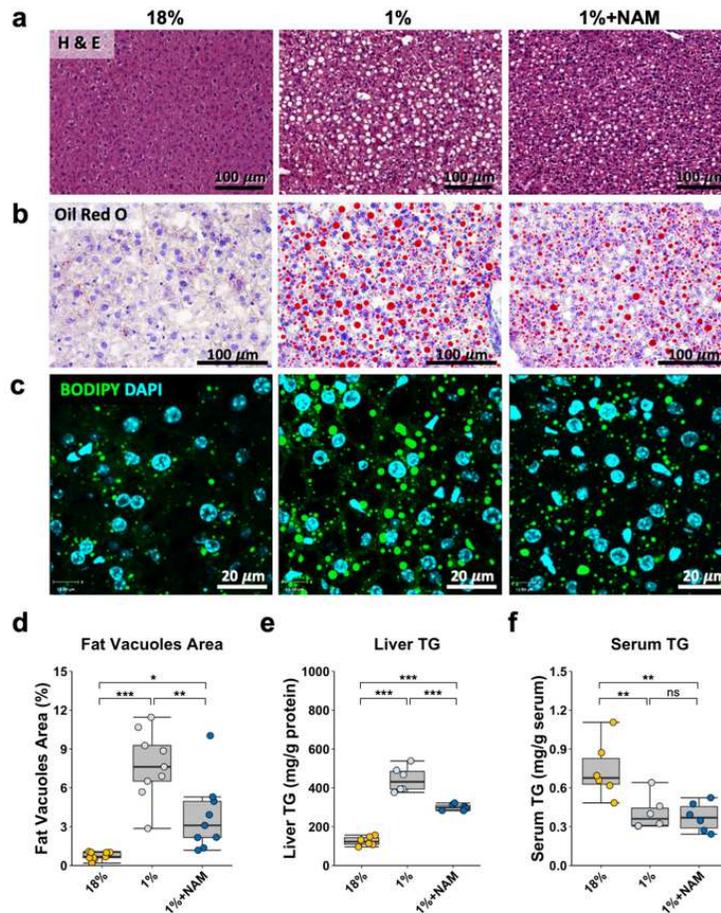
652 **Supplementary Information** is available for this paper.

653 **Correspondence** and requests for materials should be addressed to R.H.J.B.



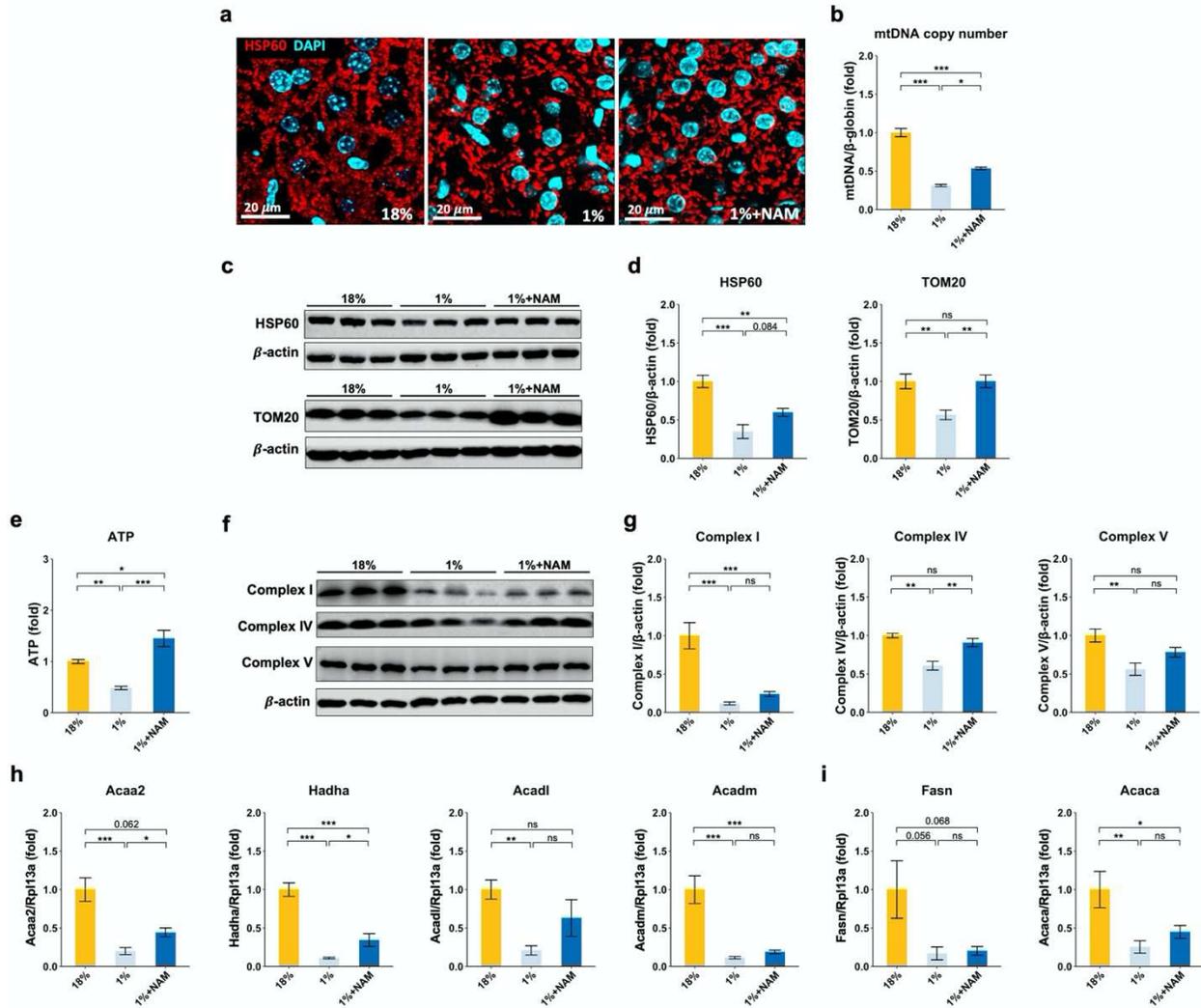
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655 **Fig. 1 Feeding a 1% protein diet with or without NAM supplementation on basic animal**
 656 **characteristics.** **a** Experimental design. **b** Average food and liquid intake during day 7 to day 14
 657 (n=7 for 18%; n=5 for 1% and 1%+NAM). **c** Body weight change throughout experiment (n=15).
 658 **d** Final body weight and body length assessed on day 14 (n=15). **e** Liver weight and liver
 659 weight/body weight ratio (n=12 for 18%; n=10 for 1% and 1%+NAM). **f** Fasting glucose levels
 660 (n=7 for 18%; n=8 for 1%; n=7 for 1%+NAM). **g** Respiratory exchange ratio (RER) and energy
 661 expenditure (n=7 for 18%; n=6 for 1%; n=7 for 1%+NAM). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns
 662 as not significant, one-way ANOVA followed by Tukey's post hoc test. Data are shown as the
 663 mean \pm S.E.M.



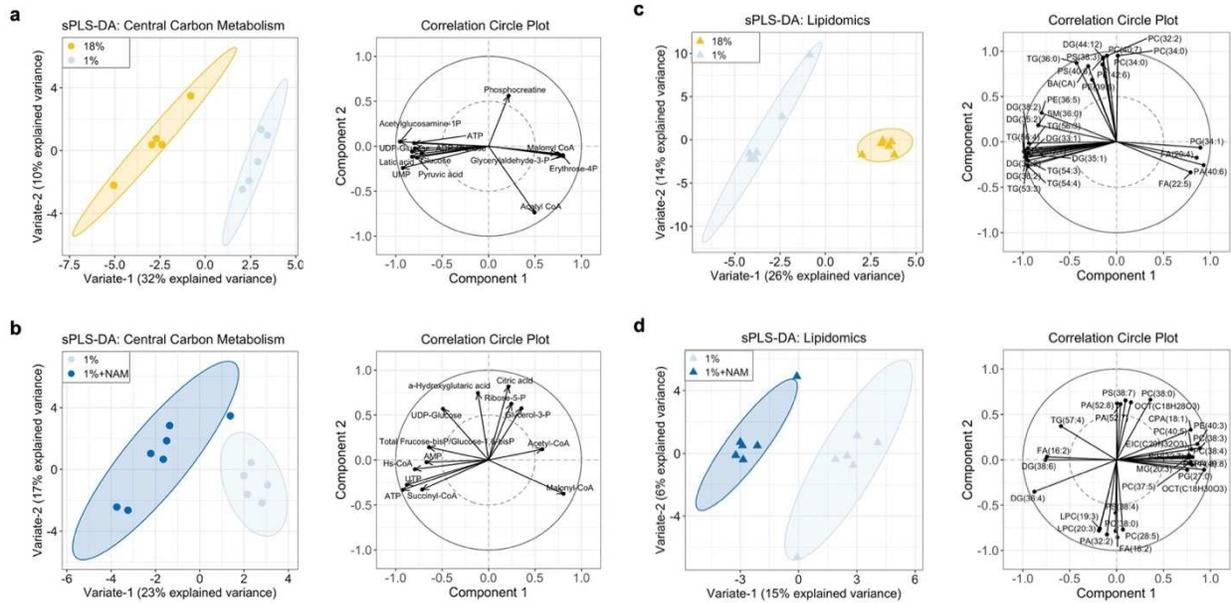
664

665 **Fig. 2 The effect of 1% protein feeding with or without NAM supplementation on hepatic**
 666 **lipid accumulation.** **a** Representative hematoxylin and eosin staining images of the liver (20X
 667 magnification). Cytoplasm was stained in red, and nucleus was stained in purple. **b** Representative
 668 oil red o stain staining images of the liver (20X magnification). Fat droplet was stained in red, and
 669 nucleus was stained in purple. **c** Representative immunofluorescence images of the liver (40X
 670 magnification). BODIPY was used to stain fat droplet in green, and DAPI was used to counter
 671 stain nucleus in blue. **d** Quantification of fat vacuoles area (n=9). **e** Liver TG concentrations (n=6).
 672 **f** Serum TG concentrations (n=6). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns as not significant, one-
 673 way ANOVA followed by Tukey's post hoc test. Data are shown as the mean \pm S.E.M. Scale bars
 674 are as indicated.



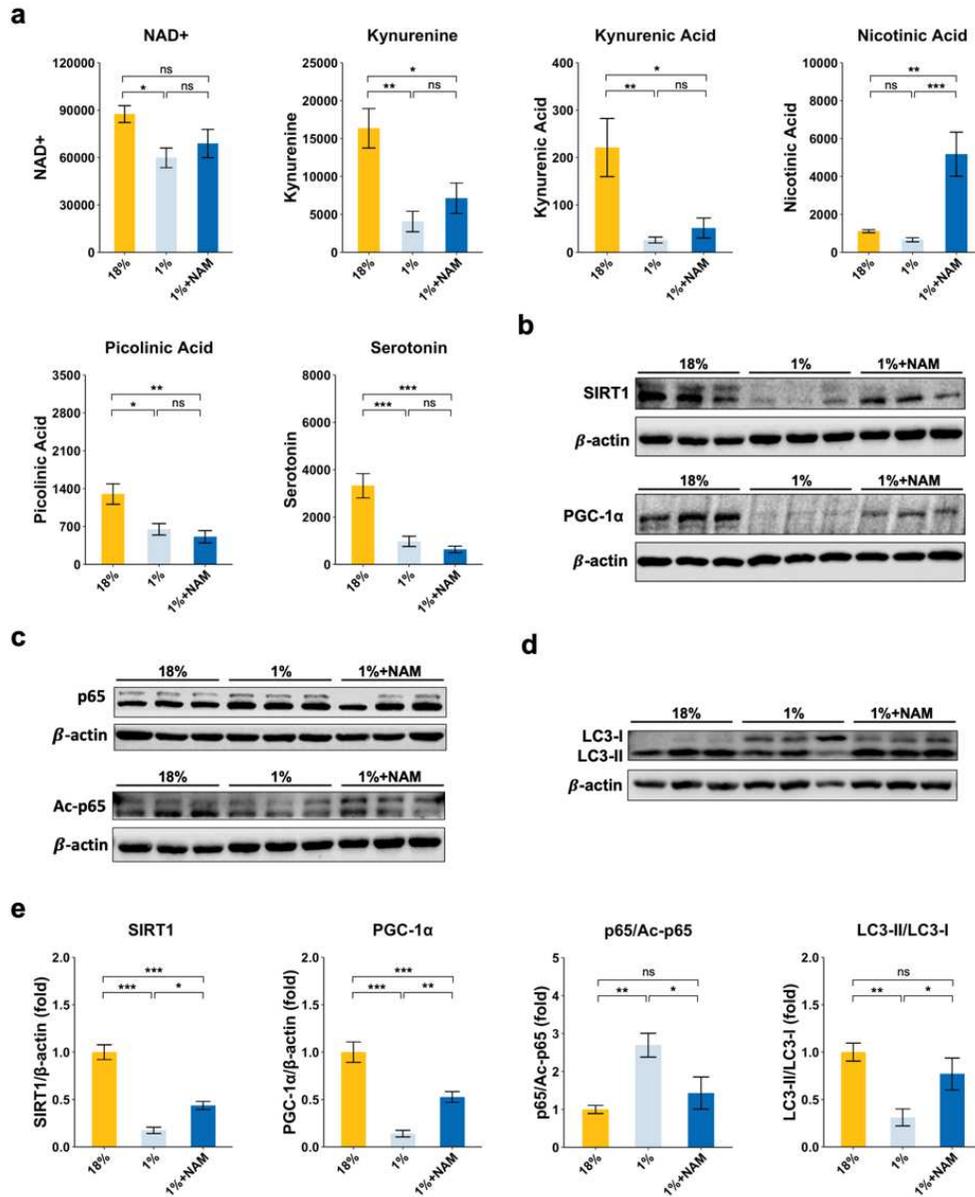
675

676 **Fig. 3 The effect of NAM supplementation on mitochondrial characteristics of 1% protein**
 677 **fed model. a** Representative immunofluorescence images of mitochondrial (60X magnification).
 678 HSP60 was used to stain mitochondrial in red, and DAPI was used to counter stain nucleus in blue.
 679 **b** mtDNA copy number (n=6). **c, d** Western blots and quantification of HSP60 and TOM20 (n=3).
 680 **e** ATP levels (n=11 for 18% and 1%; n=7 for 1%+NAM). **f, g** Western blots and quantification of
 681 complex I, complex IV and complex V (n=3). **h** mRNA expression of β -oxidation genes (n=6). **i**
 682 mRNA expression of lipid genesis genes (n=6). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns as not
 683 significant, one-way ANOVA followed by Tukey's post hoc test. Data are shown as the mean \pm
 684 S.E.M. Scale bars are as indicated.



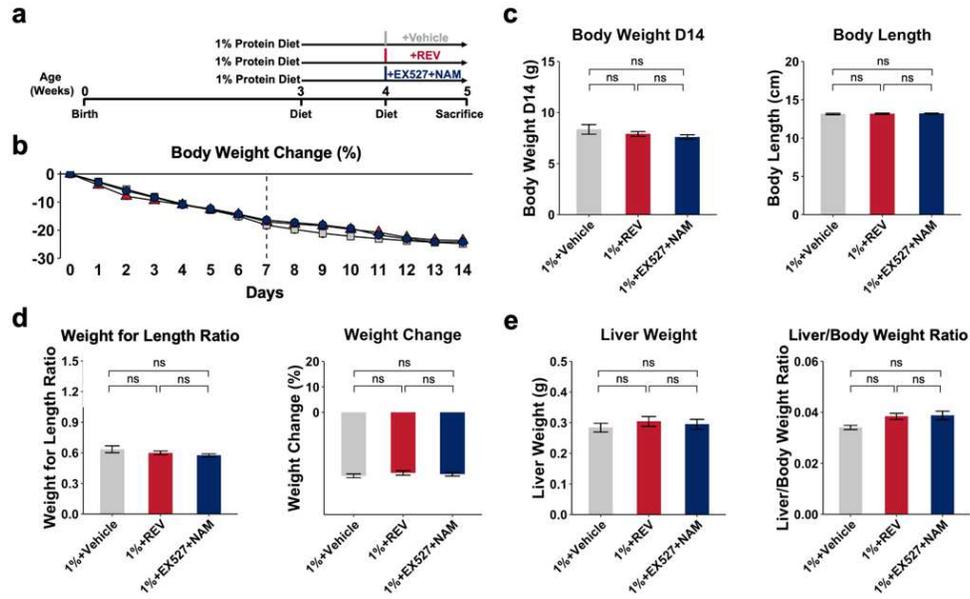
685

686 **Fig. 4** Hepatic metabolomic and lipidomic profiles under 18% protein diet, 1% protein diet,
 687 and 1% protein diet with NAM supplementation. **a** sPLS-DA and correlation circle plots of
 688 hepatic central carbon metabolism showing separation of 18% and 1% protein diet group (n=5). **b**
 689 sPLS-DA and correlation circle plots of hepatic central carbon metabolism showing separation of
 690 1% protein diet and NAM treated group (n=5 for 1%; n=7 for 1%+NAM). **c** sPLS-DA and
 691 correlation circle plots of hepatic lipidomics showing separation of 18% and 1% protein diet group
 692 (n=6). **d** sPLS-DA and correlation circle plots of hepatic lipidomics showing separation of 1%
 693 protein diet and NAM treated group (n=6).



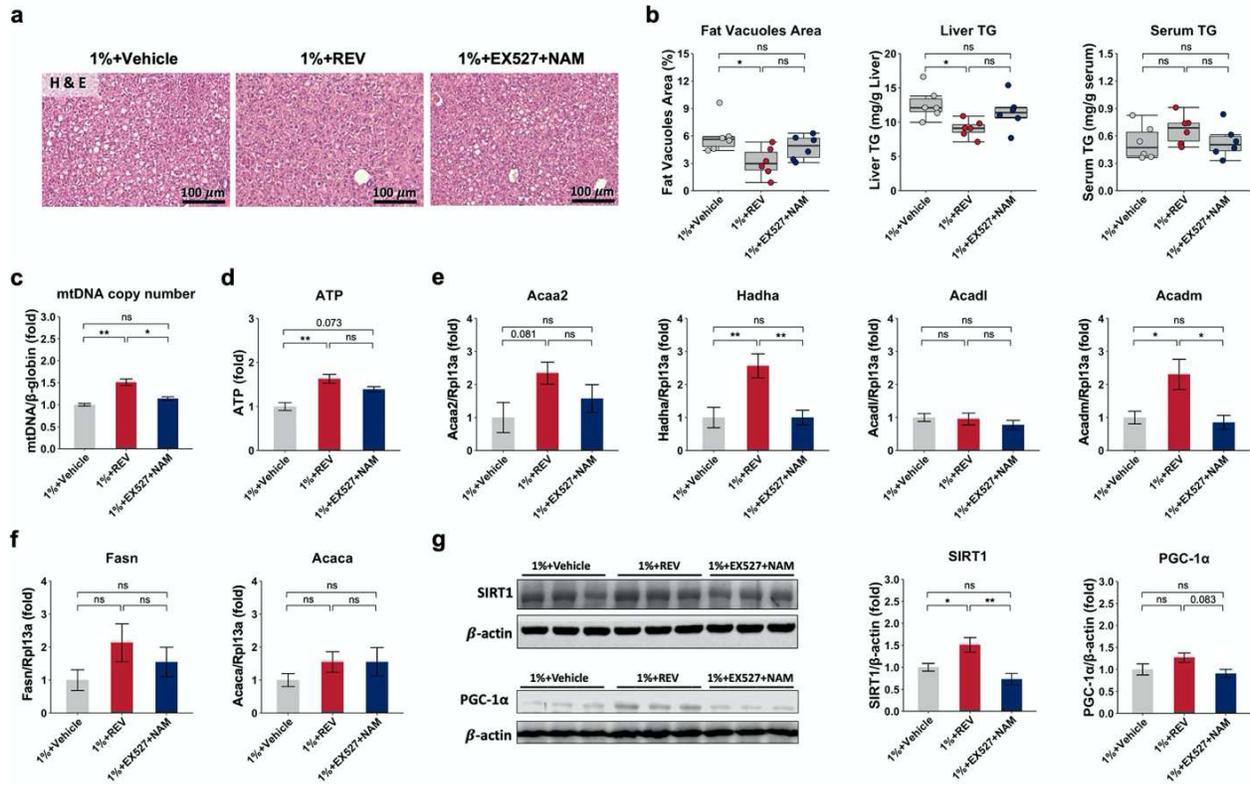
694

695 **Fig. 5 The effect of 1% protein feeding with or without NAM supplementation on TRP-NAM**
 696 **pathway metabolites, SIRT1 and downstream targets, and autophagy levels. a** Hepatic NAD+
 697 levels and TRP-NAM pathway metabolites (n=6). **b** SIRT1 and PGC-1α western blots (n=3). **c**
 698 p65 and Acetyl-p65 western blots (n=3). **d** Autophagy markers LC3 western blots (n=3). **e**
 699 Quantification of protein levels in western blots. *p < 0.05, **p < 0.01, ***p < 0.001, ns as not
 700 significant, one-way ANOVA followed by Tukey's post hoc test. Data are shown as the mean ±
 701 S.E.M.



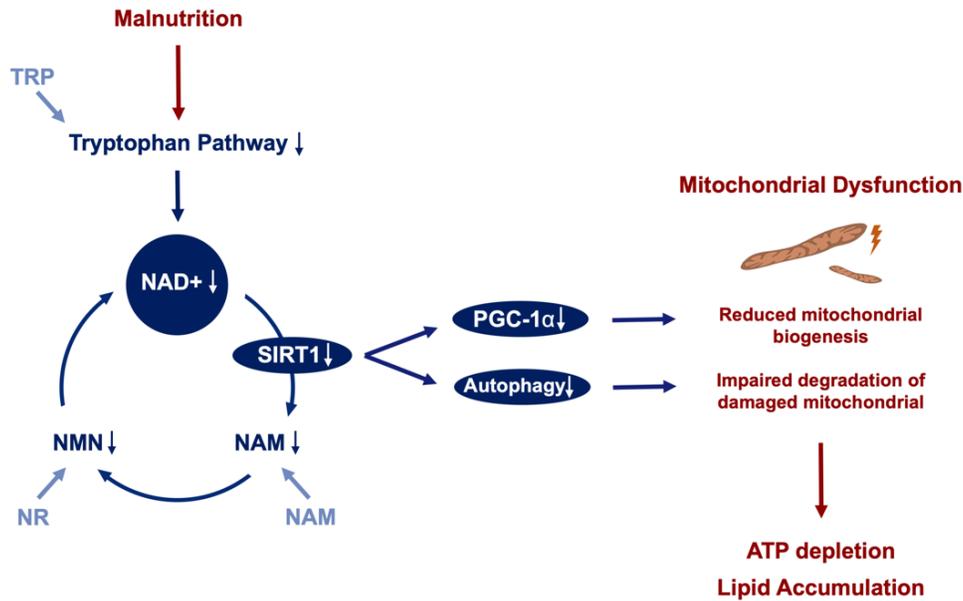
702

703 **Fig. 6 The effect of SIRT1 modulators on basic animal characteristics.** **a** Experiment design.
 704 **b** Body weight change throughout experiment (n=6). **c** Average food and liquid intake during day
 705 7 to day 14 (n=6). **d** Final body weight, body length, and weight for length ratio assessed at day
 706 14 (n=6). **e** Liver weight, liver weight to body weight ratio (n=6). *p < 0.05, **p < 0.01, ***p <
 707 0.001, ns as not significant, one-way ANOVA followed by Tukey's post hoc test. Data are shown
 708 as the mean ± S.E.M.



709

710 **Fig. 7 The effect of SIRT1 modulators on hepatic steatosis, mitochondrial characteristics,**
 711 **SIRT1 and its downstream targets.** **a** Representative hematoxylin and eosin staining images of
 712 the liver (20X magnification). Cytoplasm was stained in red, and nucleus was stained in purple. **b**
 713 Quantification of liver histology and TG levels (n=6). **c** mtDNA copy number (n=6). **d** ATP levels
 714 (n=6). **e** mRNA expression of β -oxidation genes (n=6). **f** mRNA expression of lipid genesis genes
 715 (n=6). **g** SIRT1 and PGC-1 α western blots and quantification (n=3). * p < 0.05, ** p < 0.01, *** p <
 716 0.001, ns as not significant, one-way ANOVA followed by Tukey's post hoc test. Data are shown
 717 as the mean \pm S.E.M. Scale bars are as indicated.



718

719 **Fig. 8 Proposed model of the role of the TRP-NAM pathway in malnutrition-induced hepatic**
 720 **metabolic disturbances.** In protein malnutrition, decreased TRP availability will decrease the
 721 kynurenine pathway activity, which is associated with NAD⁺ and NAM deficiency. This would
 722 disturb NAD⁺ salvage pathway, including SIRT1, influence its downstream target PGC-1 α and
 723 autophagy, which affect mitochondrial quality and function. These changes lead to ATP depletion
 724 and lipid accumulation in the liver. We hypothesize that supplement with TRP-NAM modulator
 725 would influence NAD⁺ salvage pathway. This would thereby activate SIRT1, influence PGC-1 α
 726 deacetylation and autophagy, which will have a positive effect on mitochondrial health, affect
 727 mitochondrial biogenesis and clearance of damaged mitochondrial, then improve ATP generation
 728 and reduce lipid accumulation in the liver.

Figures

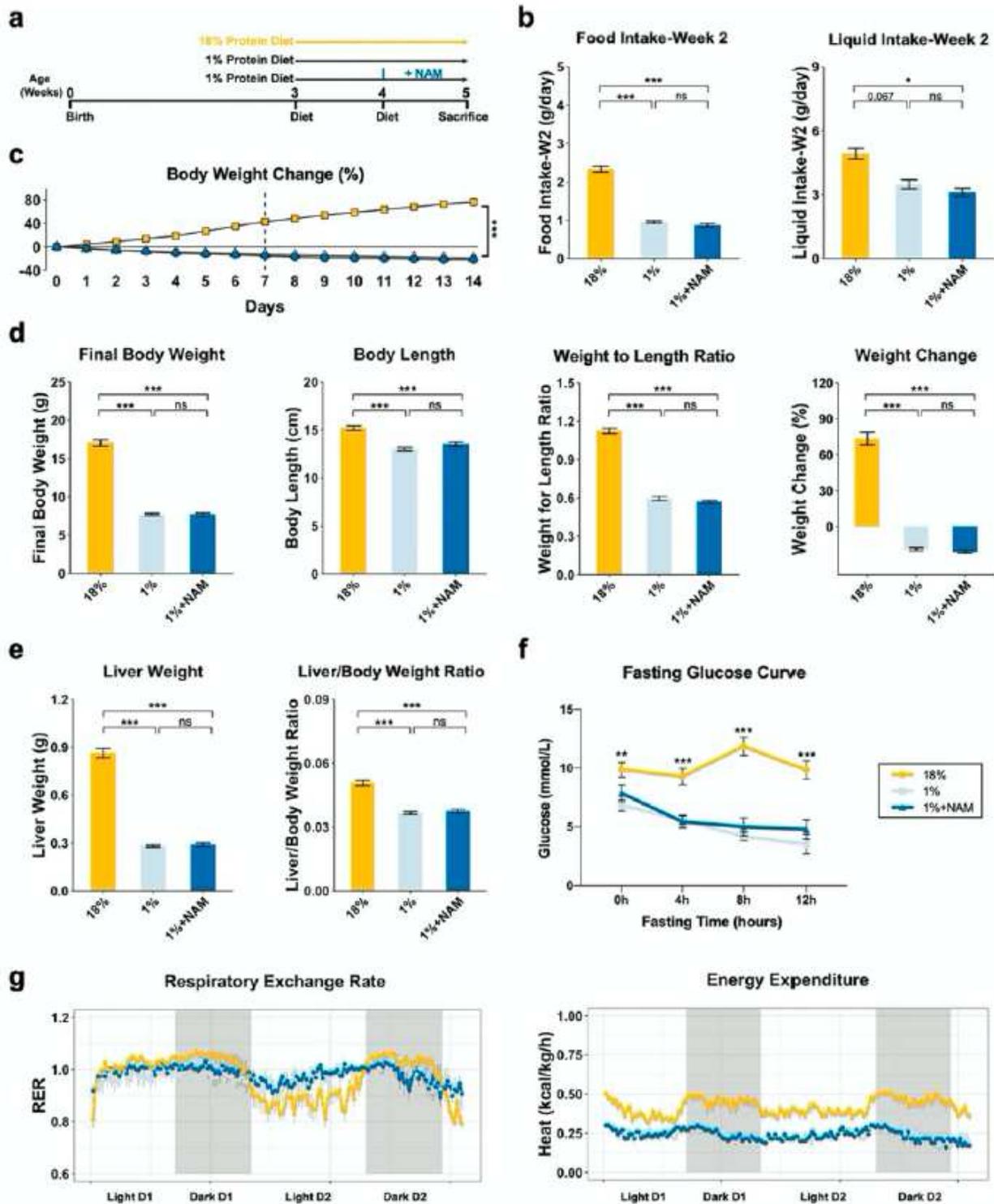


Figure 1

Feeding a 1% protein diet with or without NAM supplementation on basic animal characteristics. a Experimental design. b Average food and liquid intake during day 7 to day 14 (n=7 for 18%; n=5 for 1% and 1%+NAM). c Body weight change throughout experiment (n=15). d Final body weight and body

length assessed on day 14 (n=15). e Liver weight and liver weight/body weight ratio (n=12 for 18%; n=10 for 1% and 1%+NAM). f Fasting glucose levels (n=7 for 18%; n=8 for 1%; n=7 for 1%+NAM). g Respiratory exchange ratio (RER) and energy expenditure (n=7 for 18%; n=6 for 1%; n=7 for 1%+NAM). *p < 0.05, **p < 0.01, ***p < 0.001, ns as not significant, one-way ANOVA followed by Tukey's post hoc test. Data are shown as the mean \pm S.E.M.

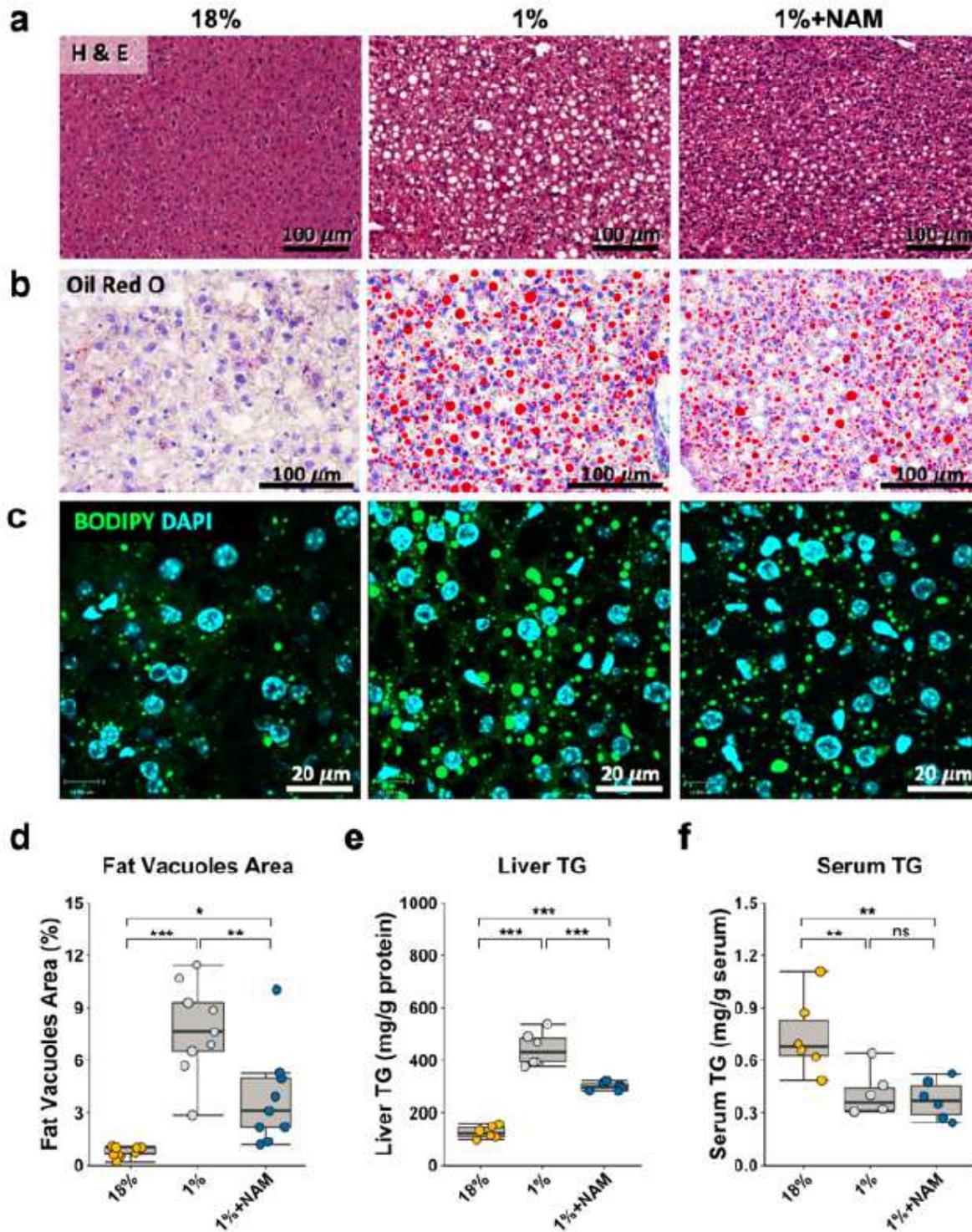


Figure 2

The effect of 1% protein feeding with or without NAM supplementation on hepatic lipid accumulation. a Representative hematoxylin and eosin staining images of the liver (20X magnification). Cytoplasm was stained in red, and nucleus was stained in purple. b Representative oil red o stain staining images of the liver (20X magnification). Fat droplet was stained in red, and nucleus was stained in purple. c Representative immunofluorescence images of the liver (40X magnification). BODYPY was used to stain fat droplet in green, and DAPI was used to counter stain nucleus in blue. d Quantification of fat vacuoles area (n=9). e Liver TG concentrations (n=6). f Serum TG concentrations (n=6). *p < 0.05, **p < 0.01, ***p < 0.001, ns as not significant, one way ANOVA followed by Tukey's post hoc test. Data are shown as the mean \pm S.E.M. Scale bars are as indicated.

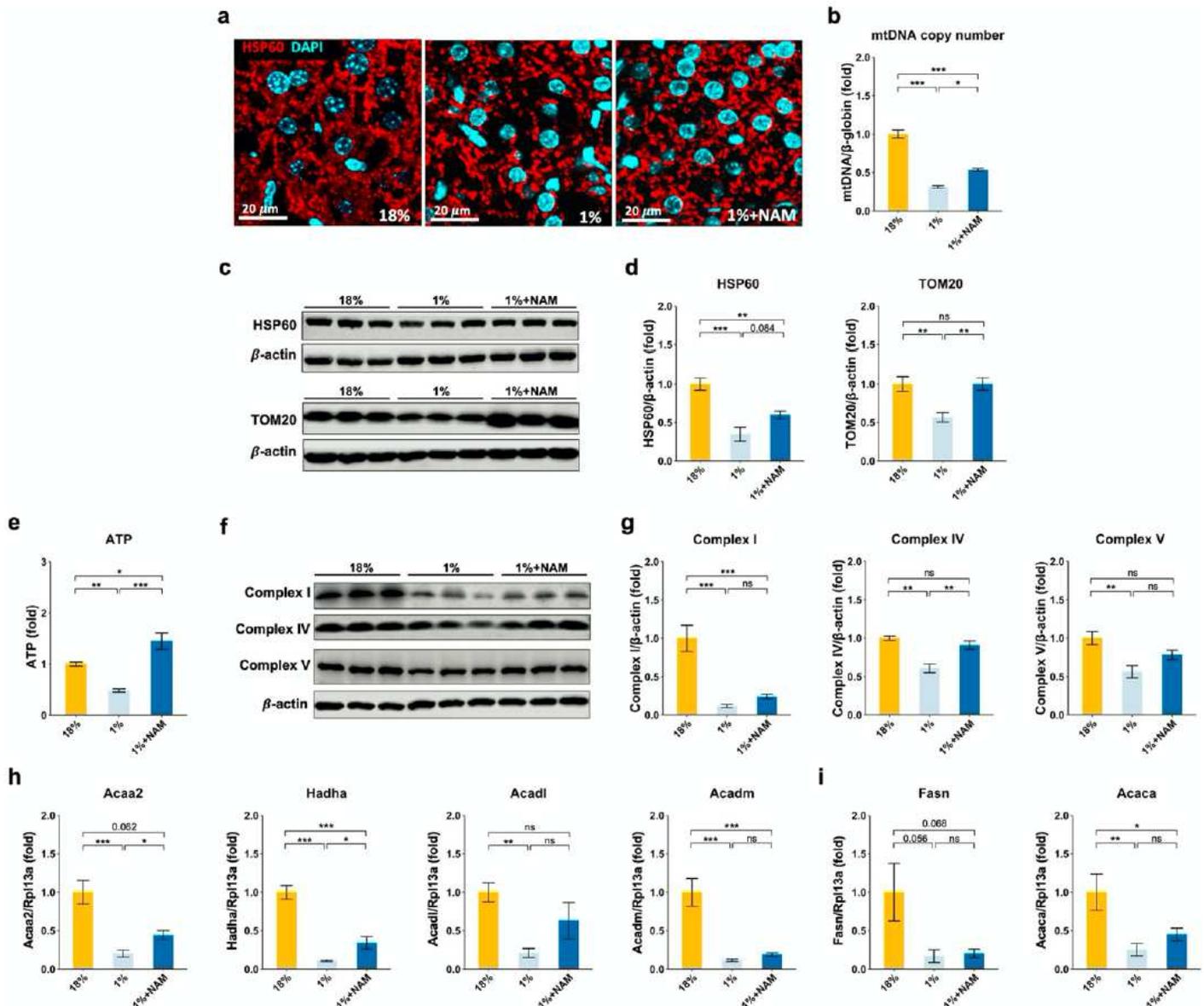


Figure 3

The effect of NAM supplementation on mitochondrial characteristics of 1% protein fed model. a Representative immunofluorescence images of mitochondrial (60X magnification). HSP60 was used to stain mitochondrial in red, and DAPI was used to counter stain nucleus in blue. b mtDNA copy number

(n=6). c, d Western blots and quantification of HSP60 and TOM20 (n=3). e ATP levels (n=11 for 18% and 1%; n=7 for 1%+NAM). f, g Western blots and quantification of complex I, complex IV and complex V (n=3). h mRNA expression of β -oxidation genes (n=6). i mRNA expression of lipid genesis genes (n=6). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns as not significant, one-way ANOVA followed by Tukey's post hoc test. Data are shown as the mean \pm S.E.M. Scale bars are as indicated.

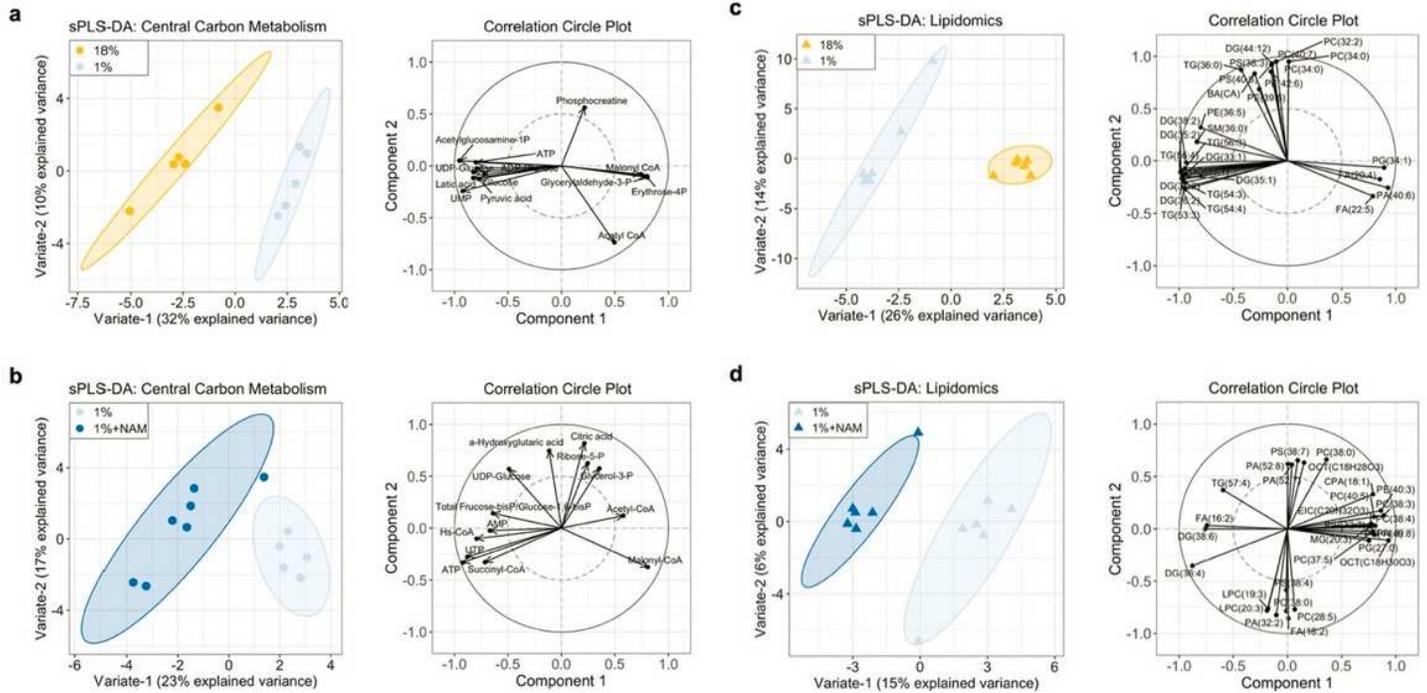


Figure 4

Hepatic metabolomic and lipidomic profiles under 18% protein diet, 1% protein diet, and 1% protein diet with NAM supplementation. a sPLS-DA and correlation circle plots of hepatic central carbon metabolism showing separation of 18% and 1% protein diet group (n=5). b sPLS-DA and correlation circle plots of hepatic central carbon metabolism showing separation of 1% protein diet and NAM treated group (n=5 for 1%; n=7 for 1%+NAM). c sPLS-DA and correlation circle plots of hepatic lipidomics showing separation of 18% and 1% protein diet group (n=6). d sPLS-DA and correlation circle plots of hepatic lipidomics showing separation of 1% protein diet and NAM treated group (n=6).

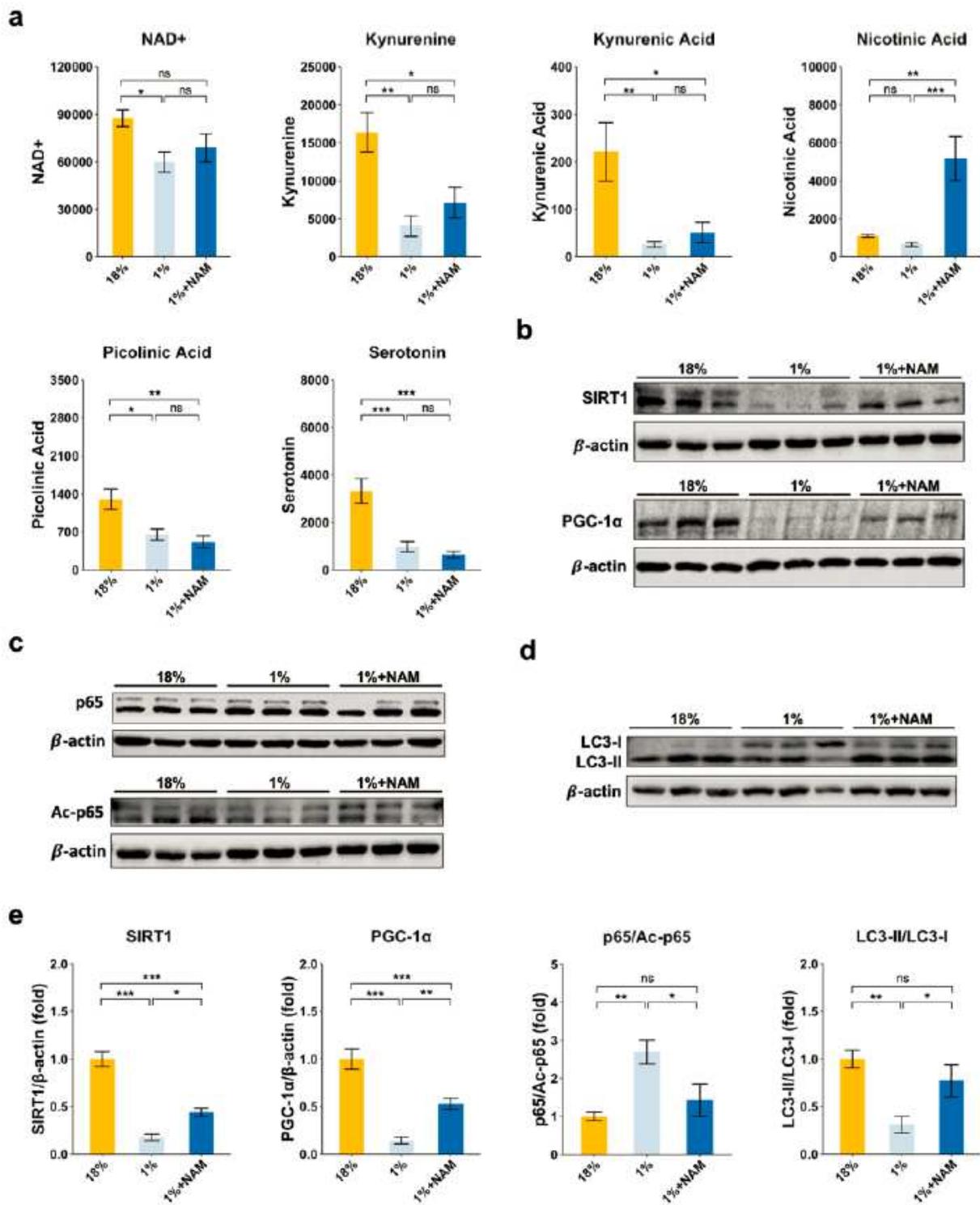


Figure 5

The effect of 1% protein feeding with or without NAM supplementation on TRP-NAM pathway metabolites, SIRT1 and downstream targets, and autophagy levels. a Hepatic NAD⁺ levels and TRP-NAM pathway metabolites (n=6). b SIRT1 and PGC-1 α western blots (n=3). c p65 and Acetyl-p65 western blots (n=3). d Autophagy markers LC3 western blots (n=3). e Quantification of protein levels in western blots.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns as not significant, one-way ANOVA followed by Tukey's post hoc test. Data are shown as the mean \pm S.E.M.

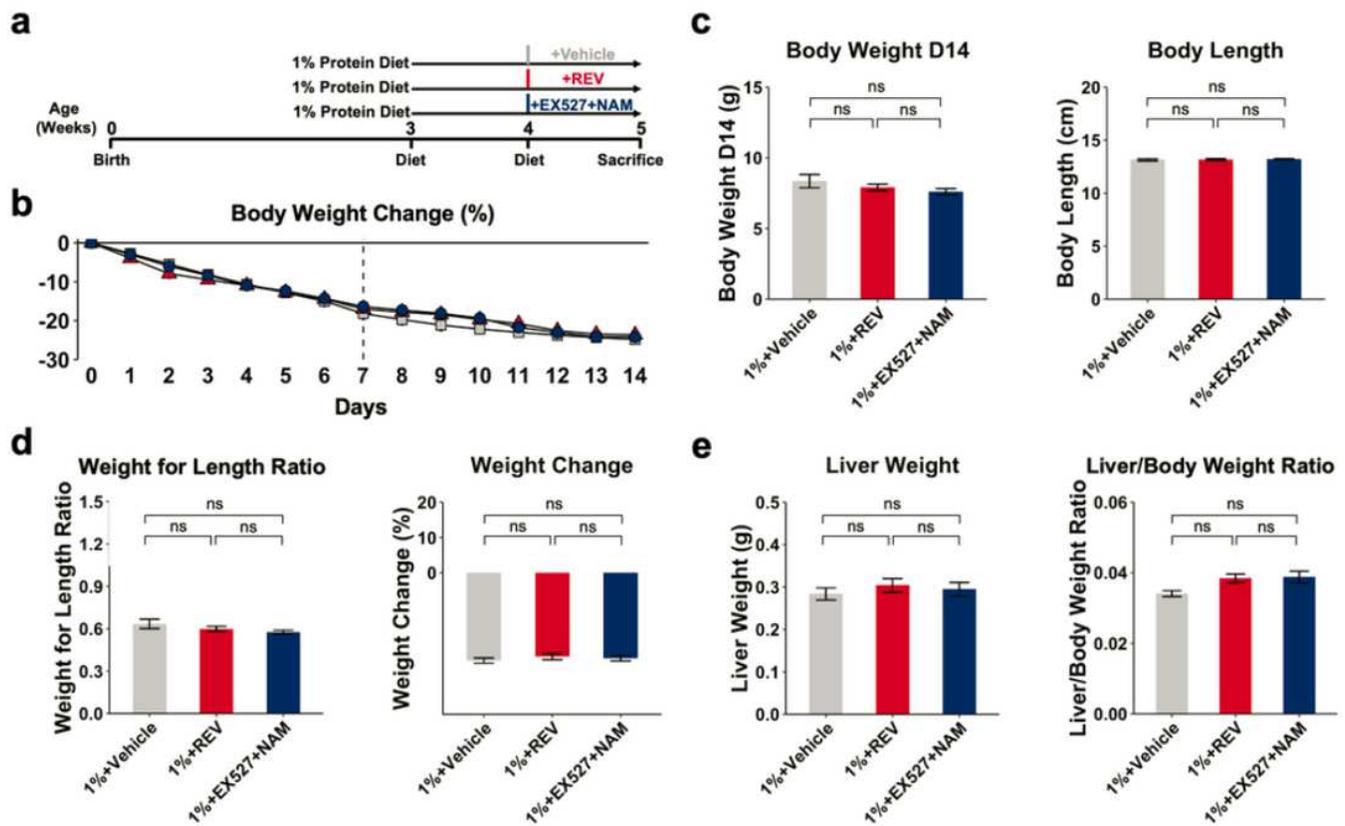


Figure 6

The effect of SIRT1 modulators on basic animal characteristics. a Experiment design. b Body weight change throughout experiment (n=6). c Average food and liquid intake during day 7 to day 14 (n=6). d Final body weight, body length, and weight for length ratio assessed at day 14 (n=6). e Liver weight, liver weight to body weight ratio (n=6). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns as not significant, one-way ANOVA followed by Tukey's post hoc test. Data are shown as the mean \pm S.E.M.

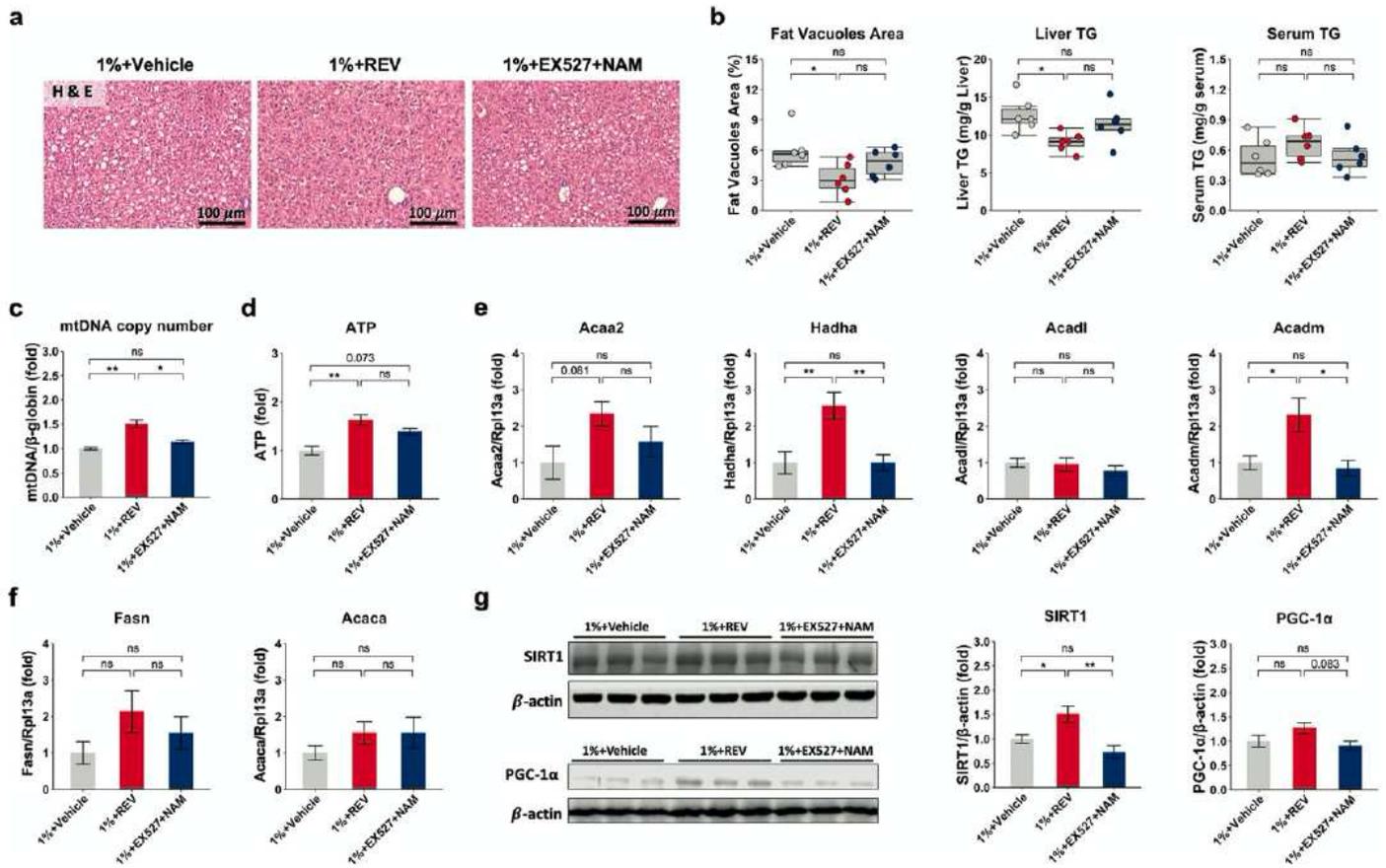


Figure 7

The effect of SIRT1 modulators on hepatic steatosis, mitochondrial characteristics, SIRT1 and its downstream targets. a Representative hematoxylin and eosin staining images of the liver (20X magnification). Cytoplasm was stained in red, and nucleus was stained in purple. b Quantification of liver histology and TG levels (n=6). c mtDNA copy number (n=6). d ATP levels (n=6). e mRNA expression of β -oxidation genes (n=6). f mRNA expression of lipid genesis genes (n=6). g SIRT1 and PGC-1 α western blots and quantification (n=3). *p < 0.05, **p < 0.01, ***p < 0.001, ns as not significant, one-way ANOVA followed by Tukey's post hoc test. Data are shown as the mean \pm S.E.M. Scale bars are as indicated.

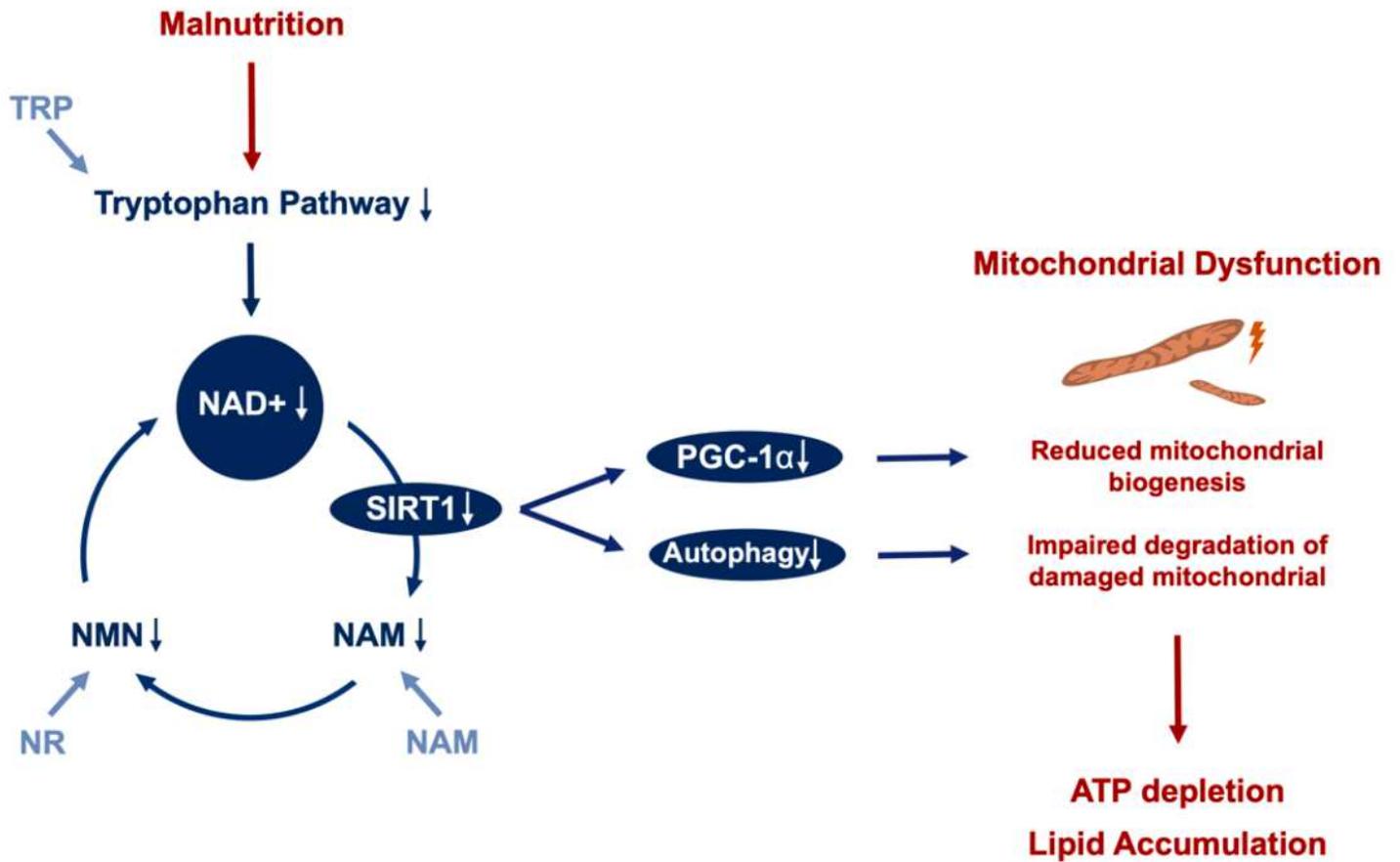


Figure 8

Proposed model of the role of the TRP-NAM pathway in malnutrition-induced hepatic metabolic disturbances. In protein malnutrition, decreased TRP availability will decrease the kynurenine pathway activity, which is associated with NAD⁺ and NAM deficiency. This would disturb NAD⁺ salvage pathway, including SIRT1, influence its downstream target PGC-1 α and autophagy, which affect mitochondrial quality and function. These changes lead to ATP depletion and lipid accumulation in the liver. We hypothesize that supplement with TRP-NAM modulator would influence NAD⁺ salvage pathway. This would thereby activate SIRT1, influence PGC-1 α deacetylation and autophagy, which will have a positive effect on mitochondrial health, affect mitochondrial biogenesis and clearance of damaged mitochondrial, then improve ATP generation and reduce lipid accumulation in the liver.

Supplementary Files

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- [Supplementaryinformation.pdf](#)