Maternal dietary deficiencies in folic acid and choline change metabolites levels in offspring after ischemic stroke

Fazian Anwar[1+], Mary-Tyler Mosley[1,2+], Paniz Jasbi[3,4], Jinhua Chi[3], Haiwei Gu[3], Nafisa M. Jadavji[5–7*]

[+] Authors contributed to manuscript equally.

[1]College of Osteopathic Medicine, Midwestern University, Glendale AZ

[2]Department of Human Biology, Stanford University, Stanford, CA

[3]College of Health Solutions, Arizona State University, Phoenix, AZ, US

[4]Systems Precision Engineering and Advanced Research (SPEAR), Theriome Inc., Phoenix, AZ, USA

[5] Department of Biomedical Sciences, Southern Illinois University, Carbondale, IL, USA

[6] Department of Child Health, University of Arizona, Phoenix, AZ, USA

[7] Department of Neuroscience, Carleton University, Ottawa, ON, Canada

Corresponding Author
Nafisa M. Jadavji, PhD
Department of Biomedical Sciences
Division of Physiology
SIU School of Medicine
Life Science III, room 245
1125 Lincoln Drive
Mail Code 6512
Carbondale, IL 62901
Phone: 623-226-1205
nafisa.jadavji@siu.edu
ORCID: 0000-0002-3557-7307

Keywords

One-carbon metabolism; ischemic stroke; maternal nutrition; offspring; microbiome; metabolomics

Abstract

Ischemic stroke is a debilitating disease, with nutrition being a modifiable risk factor. Changes in levels of metabolites can be used to measure the alterations in the gut, a significant marker for the etiology of diseases. This study utilized untargeted metabolomics to investigate changes in fecal samples of offspring in response to maternal dietary deficiencies and ischemic stroke. Female mice were placed on control (CD), folic acid- (FADD), or choline-deficient (ChDD) diets prior to, during pregnancy, and lactation. Offspring were weaned on to CD and at 2 months of age an ischemic stroke was induced. Fecal samples were collected prior to ischemic stroke, and at 1- and 4-weeks post-stroke for analysis. Sex and maternal dietary differences in metabolites were observed at both the 1- and 4-week post-stroke timepoints. At the 1-week post-stroke, female FADD offspring had more changes in metabolites than males. Comparatively, at the 4-week post-stroke timepoint, female offspring on either FADD or ChDD demonstrated metabolite changes. This study demonstrates a long-lasting impact of maternal dietary deficiencies on central nervous system and gut microbiome function after ischemic stroke.

Summary Statement

Our study investigated metabolite changes in female and male offspring fecal samples from mothers maintained on folic acid or choline deficient diet. We report that female offspring metabolite levels were impacted.

Introduction

Ischemic stroke is one of the leading causes of morbidity and mortality globally. The incidence of stroke in adults has been increasing due to factors such as high blood pressure, vascular disorders, diabetes, and obesity (Mozaffarian et al., 2015). By 2030, the number of

63 individuals affected by stroke is predicted to increase by 3.4 million (Ovbiagele et al., 2013). While
64 there has been a higher prevalence of ischemic stroke in older age individuals (> 65 years) (Feigin
65 et al., 2014; Mozaffarian et al., 2015), recent studies have shown that the incidence of stroke among
66 people under 50 years of age is alarmingly increasing (Feigin et al., 2014; Smajlović, 2015; Tong
67 et al., 2016). More importantly it is becoming more evident that nutrition is a well-established and
68 a modifiable risk factor for vascular dysfunction leading to ischemic stroke (Benjamin et al., 2018;
69 Hankey, 2012; Spence et al., 2017).

70 Although previous studies have established nutrition as a modifiable risk factor for vascular
71 dysfunction and ischemic stroke (Benjamin et al., 2018; Hankey, 2012; Spence et al., 2017),
72 logistical challenges in conducting longitudinal studies have limited research on the impact of
73 early-life nutrition on late-life neurological outcomes. Pregnancy and early life are critical periods
74 for neurodevelopment and lay the foundation for future brain function and health (Carolan-Olah
75 et al., 2015; Kolb et al., 2016; Pannia et al., 2016). In the US, health disparities lead many women
76 to receive inadequate vitamins and nutrients during pregnancy. For example, nearly one in three
77 African-American women do not get enough folic acid daily (Bailey et al., 2010). The same is true
78 for Spanish-speaking Mexican-American women; they often do not get enough folic acid (Hamner
79 et al., 2011). Furthermore, approximately one-fourth of women in the US do not consume enough
80 choline from their diet (Jensen et al., 2007; Zeisel, 2013), and women in low-income countries
81 often face nutritional deficiencies during pregnancy, making this a serious global health issue
82 (Gernand et al., 2016). Despite recent attention to the potential effects of maternal dietary
83 deficiency on early life and childhood development, the long-term impact of maternal dietary
84 deficiencies during pregnancy on the child's future vascular health is not yet well understood.
85 Evidence suggests that maternal nutrition plays an important role in disease onset and outcome

later in life (Di Meco & Praticò, 2019; Gaillard, 2015; Palinski, 2014; Yessoufou & Moutairou, 2011). For example, maternal obesity during pregnancy is implicated in the onset of metabolic diseases, such as diabetes in offspring(Gaillard, 2015; Yessoufou and Moutairou, 2011), but more life course studies are required (Gaillard, 2015). Furthermore, it has been shown that the maternal environment plays an important role in cardiovascular risk in offspring (Palinski, 2014). Using animal models, many groups have shown that maternal diet (e.g., high-fat diet, and caloric restriction, etc.) can modulate cerebrovascular structure and function, leading to endothelial dysfunction, reduced smooth muscle contractility, and decreased myogenic tone, all of which significantly influence peripheral and cerebral vascular health in mouse and rat adult offspring (Campbell et al., 2005; Durrant et al., 2014; Lin et al., 2018). Our group has demonstrated that maternal dietary deficiencies in folic acid or choline impact offspring ischemic stroke outcome (Clementson et al., 2023; Hurley et al., 2023; Pull et al., 2023). However, the mechanisms are not yet clear, our hypothesis is that maternal diet deficient in either folic acid or choline combined with ischemic stroke will result in changes to metabolites within the gut. The aim of the present study was to investigate changes in metabolite levels of fecal matter in male and female offspring exposed to FADD and ChDD during early neurodevelopment and an ischemic stroke during adulthood. Untargeted metabolite analysis has been shown to provide some insight to changes after stroke (Zhao et al., 2022), predict stroke outcome (Chi et al., 2021), as well as generate data for biomarkers (Li et al., 2018; M.-H. Wu et al., 2023).

Results

Maternal diet deficiencies in FADD or ChDD during pregnancy and lactation impacted metabolite levels at the 1- and 4-week post-stroke timepoints. The number of changes in metabolites because of experimental manipulations (maternal diet or offspring sex) are

summarized in Figure 1. The number of metabolites measured in offspring fecal samples was extensive. We have listed all the metabolites that were significantly difference in supplementary Table 1 with the corresponding p-values for interactions between maternal diet and offspring sex, as well as main effects of maternal diet and sex.

Figure 1. Summary of two-way ANOVA analysis demonstrating the metabolite changes based on sex, maternal diet, or interaction. (A) Demonstrate difference in one metabolite pre-stroke due to change in maternal diet. (B) Demonstrate metabolite changes based on sex, maternal diet, and combined effect of both maternal diet & sex in 1-week post-stroke with no interaction. (C) Significant and enhanced changes in metabolites seen at 4-weeks post stroke based on sex, maternal diet, or combined with 3 metabolites changes being affected differently by sex and maternal diet.

Pre-Stroke

At the pre-stroke time point, a significant difference in HEPES levels was observed between maternal diet groups ($p = 0.00052$), with female ChDD offspring having elevated levels of this metabolite (Table 1). There was also a difference between male and female offspring ($p = 0.0087$) and interaction between maternal diet and sex of animals ($p < 0.0001$).

One-Week Post-Stroke

Interactions between maternal diet and sex

There were interactions between maternal diet and offspring sex in PALDA (Table 2; p = 0.213), 3-Oxopalmitic Acid (p = 0.001), 7-Dehydrocholesterol (p = 0.001), Tetrahydrodeoxycorticosterone (p = 0.001), and Avenasterol (p = 0.011).

Sex differences

Sex differences were observed in in PALDA (Table 2, p = 0.0307) and N6-(2-1Himidazol-4-yl) ethyl) lysine (p = 0.0221). Significant sex differences were followed up with pairwise comparisons, details are listed in Table 2.

Impact of Maternal Diet

After one-week post-stroke we observed maternal dietary differences in fecal measurements for the following metabolites, PALDA (Table 2; p = 0.0006), N6-(2-1Himidazol-4-yl) ethyl)lysine (p = 0.001), 3-Oxopalmitic Acid (p = 0.017), 7-Dehydrocholesterol (p = 0.0001), Yakkasterone (p = 0.0001), Tetrahydrodeoxycorticosterone (p = 0.013), Cromide BEM (p=0.0063), certonardosterol (p = 0.0001), 4betamethylzymosterol-4-carbaldehyde (p = 0.001), 4a,10-Dihydroxy-2,2,6a,6b,9,9,12a-heptamethyl-2,3,4,4a,6,6a,6b (p = 0.04), 3-dehydro-6-deoxoteasterone (p = 0.001), 2-Aminoethyl(2R)-3-[(1Z)-1-h(14Z)-14-Tricosen-10-one (p = 0.0001), 2-Aminoethyl(2R)-3-[(1Z)-1-h(14Z)-14-Tricosen-10-one (p = 0.0001), 1-(3,5-Dihydroxyphenyl)-2-heptadecanone (p = 0.0001), (R)-palmiticmonoisopropanolamide (p = 0.0002), (R)-palmiticmonoisopropanolamide (p = 0.006), and (3S,5E,7E,24R)-(6,19,19-~2~H_3_)-9,10-Secocholesta-5,7,10-trie (p = 0.0001). Significant maternal diet differences in metabolites were followed up with pairwise comparisons, details are listed in table 2.

154 ***Four-Week Post-Stroke***

155 ***Interactions between maternal diet and sex***

156 There was an interaction between maternal diet and offspring sex for the following
157 metabolites, HEPES (Table 3, p = 0.032), 1-(4-Aminobutyl)urea (p = 0.0015), PRIMA-1 (p =
158 0.0005), N-lauroylglycine (p = 0.0036), N~6~,N~6~-Dimethyllysine (p = 0.0088), Misoprostol (p
159 = 0.032), Gly-Leu (p = 0.003), Chamazulene (p = 0.02), Esmolol (p = 0.013),
160 Dodecylethanolamide (p = 0.038), Creatine (p = 0.01), C2 Dihydroceramide (p = 0.003), BAR501
161 (p = 0.006), APM (p = 0.024), Adenine (p = 0.012), Acetylcadaverine (p = 0.0038),
162 Acetohydroxamic acid (p = 0.0084), 1-PP (p = 0.011), (4R)-5-Hydroxy-L-leucine (p = 0.003), and
163 (+)-castanospermine (p = 0.028).

164 ***Sex Differences***

165 There were sex differences in the following metabolites, Fenamole (Table 3, p = 0.03), n-
166 Hexanamide (p = 0.0019), N-Acetyl-Lglutamic Acid (p = 0.051), Sphinganine (p = 0.02),
167 Pyrrolidine (p = 0.04), Propoxur (p = 0.006), Phytosphingosine (p = 0.012), HMMNI (p = 0.0014),
168 Chamazulene (p = 0.05), Esmolol (p = 0.025), and 5-Hydroxytryptophol (p = 0.006). There were
169 interactions between maternal diet and sex in the following metabolites, n-Hexanamide (p =
170 0.0073), N-Acetyl-Lglutamic Acid (p = 0.0029), Homocitrulline (p = 0.0001), Sphinganine (p =
171 0.018), R-(+)-Etiracetam (p = 0.0005), and Pyrrolidine (p = 0.01). Significant sex differences
172 between females and males were followed up with pair wise comparisons, details are listed in table
173 4.

174 ***Impact of Maternal Diet***

175 At four weeks post-stroke there were differences in offspring metabolites, as a result of
176 maternal diet in the following: HEPES (Table 3, p = 0.0072), Fenamole (p = 0.03), 2,3-

177 Dihydroxypropylstearate ($p = 0.0004$), JWH 133 ($p < 0.0001$), Sphinganine ($p = 0.053$), 1-(4-
178 Aminobutyl)urea ($p = 0.025$), Propoxur ($p = 0.003$), phytosphingosine ($p = 0.02$), N-lauroylglycine
179 ($p = 0.021$), nicaraven ($p = 0.003$), N~6~,N~6~-Dimethyllysine ($p = 0.049$), Misoprostol ($p =$
180 0.036), lovastatin ($p = 0.0081$), Gly-Leu ($p=0.026$), Chamazulene ($p = 0.02$), Esmolol ($p = 0.0364$),
181 Dodecylethanolamide ($p = 0.009$), BAR501($p = 0.042$), APM ($p = 0.038$), 5-Hydroxytryptophol
182 ($p = 0.003$), and (+)-castanospermine ($p = 0.026$). Significant differences in metabolite levels as
183 a result of maternal diet was followed up with pairwise comparisons, details are listed in table 5.

184 **Discussion**

185 Ischemic stroke is a major health concern, with nutrition being a modifiable risk factor. We
186 have previously demonstrated offspring from female mice deficient in either folic acid or choline
187 have worse outcome after stroke (Clementson et al., 2022; Hurley et al., 2023; Pull et al., 2023) .
188 Metabolites are important marker to measure the changes in the gut microbiota, which can be
189 significant markers for different pathophysiological diseases in the body. We collected fecal
190 samples from female and male offspring whose mothers were maintained on either FADD, ChDD,
191 and CD. The fecal samples were collected at three timepoints including prior to, 1, and 4 weeks
192 post-ischemic stroke to measure metabolites using untargeted analyses. This untargeted
193 metabolomics analysis revealed a wide spectrum of detrimental effects caused by stroke and
194 maternal diet deficiencies in 3-month-old offspring. Our analysis revealed that maternal folic acid
195 dietary deficiency has significant impact on microbiota of offspring after ischemic stroke. The
196 results demonstrate that sex and maternal diet impact metabolites levels after ischemic stroke and
197 may impact outcomes.

198 At pre-stroke stage we observed changes in levels of HEPES because of maternal diet. The
199 role and involvement of these metabolites remain unclear. The differences in maternal diet and sex
200 impact on the stroke outcome become more prominent in 1-and 4-week post-stroke time points.
201 A total of 17 metabolites differences observed in different diet groups at 1 week post stroke.
202 FADD mice had lower level of metabolites compared to ChDD and CD, while ChDD mice had
203 similar metabolites level to CD mice. Male mice had lower levels of metabolites at 1 week post
204 stroke. Most of the observed metabolites observed in lower levels are involved in various survival
205 and developmental cellular mechanisms including cholesterol biogenesis, cell division,
206 macrophage polarization, and neuroprotection. Tertahydrodeoxycorticsterone, a neurosteroid,
207 have been observed to play a role in neuroprotection and neural analgesic affects (Kang et al.,
208 2021; Márk & Paragh, 2007; Mediratta et al., 2001; Patchev et al., 1997; Piette, 2020; Rupprecht,
209 1997; Wetzel et al., 1999; Womack et al., 2006). Another metabolite, 7-dehydrocholesterol
210 reductase (DHCR7) is an enzyme that catalyzes 7-dehydrocholesterol (7-DHC) to cholesterol, a
211 final step in the biogenesis of cholesterol (Huang et al., 2017; Waterham & Wanders, 2000; Xiao
212 et al., 2020). DHCR7 mutations in humans cause a clinical autosomal recessive genetic disease,
213 namely Smith-Lemli-Opitz syndrome (SLOS), which is characterized with multiple abnormalities
214 including growth deficiency, intellectual disability, and frequent infections(Honda et al., 1998).
215 JWH 133 activates cannabinoid 2 receptors in reduced ovarian ischemia-reperfusion injury due to
216 its antioxidant and anti-inflammatory effects (Aydogan Kirmizi et al., 2021; López-Dyck et al.,
217 2017; Onat et al., 2021; Wojcieszak et al., 2016). JWH-133 showed anti-obesity effects that
218 ameliorated pro-inflammatory M1 macrophage polarization through the Nrf2/HO-1 pathway (Q.
219 Wu et al., 2020). The lower levels of the involved metabolites seem to have effects on
220 neuroprotective mechanism during neurodevelopment in early age as well as have effects on the

221 immune systems and fatty acid mechanism. Dysfunction in such metabolites can also signal
222 towards growth deficiency and more prone to infections. However, the mechanism of the effects
223 remains unclear and require additional investigation.

224 At 4-week post stroke, metabolites significantly lower compared to one-week timepoint
225 are observed to have come back to normal levels while new metabolites were found to be in
226 abnormal levels. Female mice were affected more adversely with lower levels of all the metabolites
227 found in 4-week post stroke compared to pre-stroke levels. Mice on either ChDD or FADD were
228 both at lower levels as compared mice of CD. Homocitrulline levels were lower in both genders
229 on FADD diet which plays role in cell viability and mitochondrial function in menadione-treated
230 astrocytes (Zanatta et al., 2016). N-Acetyl-glutamic acid, Noradrenaline, and Creatine which play
231 roles in liver ureagenesis, cognitive process, and cellular metabolism essential for cognitive
232 abilities respectively were found in lover levels (Caldovic et al., 2010; Harper et al., 2009;
233 Prokopová, 2010; Sumien et al., 2018). Other metabolites, Dehydrocostus which have antibacterial
234 activity by inhibiting LPS-induced production of proinflammatory mediators were in lower levels
235 (Nie et al., 2019). Metabolite which are indicators of central nervous system health and play
236 protective roles were detected at lower levels in female with both FADD and CHDD diet. Some
237 of the metabolites are (+)-Castanospermine involved in reduces inflammation in brain, 5-
238 Hydroxytryptophol responsible for elicits cerebro arterial contractions (Saul et al., 1983; Tharappel
239 et al., 2020), Acetylcadaverine lower levels is markedly found Alzheimer's Disease (Paik et al.,
240 2006), BAR501 responsible for reversing intestinal inflammation and shifts activation of intestinal
241 macrophages reducing expression of inflammatory genes (Biagioli et al., 2017). C2
242 Dihydroceramide associated with increased apoptosis in human leukemia HL-60 cells (Shikata et
243 al., 2003), and Gly-Leu, Glycine acts as a neurotransmitter in central nervous system; antioxidant,

244 anti-inflammatory, cryoprotective, and immunomodulatory in peripheral and nervous tissues and
245 leucine essential amino acid used in ATP generation, protein synthesis, tissue regeneration, and
246 metabolism (Pedroso et al., 2015; Razak et al., 2017).

247 Overall, after 4-week post stroke, it is strongly evident that female offspring that come
248 from mothers maintained on FADD or ChDD have more changes in their metabolites compared
249 to males. The changes are magnified at the four-week timepoint despite being on CD compared to
250 1 week post stroke. The abnormal metabolites levels suggest that there is significant inflammation
251 found both in brain and gut. It is indicated that the body's anti-inflammatory and defense system
252 is also highly affected. Interestingly, markers for Alzheimer's disease, cancers, gut, and brain
253 inflammation were found to be changed suggesting that these subjects might experience an early
254 physical deterioration and hindered development. In a previous study, there was evidence of
255 phenotypical behavioral issues in mice despite any changes in the ischemic size of the brain infarct.
256 This is suggestive of activation of multiple pathophysiological mechanisms that have long lasting
257 effects on a developing body. This is the first study to report changes in fecal sample metabolites
258 after changes in maternal diet and ischemic stroke. More work is required, as the mechanism and
259 molecular pathway are not clear. Possible next steps may include sequencing samples to determine
260 changes in DNA. The present study provides insights that maternal dietary deficiencies in folic
261 acid when combined with ischemic stroke impact offspring metabolites in a sex dependent manner.

262

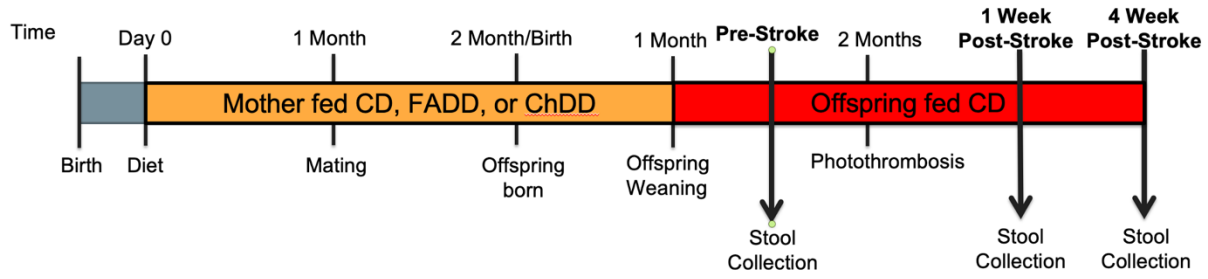
263 **Materials and methods**

264 **Animals and experimental design**

265 All experiments were conducted in accordance with the guidelines of the Midwestern
266 University Institutional Animal Care Users Committee (IACUC 2983). C57/BL6J mice (both

267 female and male) were obtained from Jackson Laboratories. The breeding pairs produced male (n
268 = 17) and female (n = 15) offspring.

269 Experimental manipulations are summarized in Figure 2. Two-month-old female mice
270 were habituated for seven days before they were placed on either control (CD), folic acid (FADD,
271 folic acid: 0.3 mg/kg), or choline deficient diets (ChDD, choline bitrate: 300 mg/kg) (Clementson
272 et al., 2023; Dam et al., 2017; Hurley et al., 2023; Jadavji, Deng, et al., 2015; Jadavji, Farr, et al.,
273 2015) (Envigo, Indianapolis, IN). The control diet contains the minimum amount of folic acid (2
274 mg/kg) and choline bitrate (1150 mg/kg). The dams were maintained on the diets 4 weeks prior to
275 pregnancy, during pregnancy, and lactation. Once female and male offspring were weaned from
276 their mothers, they were maintained on a CD. At 2 months of age, the offspring were subjected to
277 ischemic stroke, using the photothrombosis model to the sensorimotor cortex. Stool samples were
278 collected from the same offspring at three timepoints: prior to ischemic stroke, 1 week after, and
279 4 weeks after the stroke.



280 **Figure 2.** Displays the timeline of the experiment. Pregnant mothers were fed either the CD,
281 FADD, or ChDD diet throughout the months of pregnancy and lactation until the offspring
282 were weaned. Once the offspring were weaned, they were maintained on the CD. At 2 months
of age, the offspring were subjected to ischemic stroke via the PT model. Tissue and fecal
matter was collected at 1-Week Post Stroke and 4-Week Post Stroke.

283

284 **Photothrombosis Model for Ischemic Stroke**

285 At 2 months of age all female and male offspring mice were subjected to photothrombosis
286 to induce a unilateral ischemic stroke in the sensorimotor cortex. Mice were anesthetized with
287 isoflurane (1.5%) in a 70:30 nitrous oxide: oxygen mixture. Core body temperature was monitored
288 with a rectal thermometer (Harvard Apparatus, Holliston, MA) and maintained at 37 ± 0.2ºC using
289 a heating blanket. 10mg/kg of the photosensitive Rose Bengal (Sigma, St. Louis, MA) dye was
290 injected intraperitoneally 5 minutes prior to irradiation. A 532 nm green laser was placed 3 cm
291 above the animal and directed to the sensorimotor cortex (mediolateral + 0.24mm)(Abato et al.,
292 2020; Clementson et al., 2023; Hurley et al., 2023; Jadavji et al., 2017, 2019, 2019; Lee et al.,
293 2004; Mbs et al., 2023; Poole et al., 2022) for 15 min.

294 **Fecal preparation**

295 Fecal samples were collected in a microfuge tube at three timepoints, prior to, 1 and 4
296 weeks after ischemic stroke. The samples were stored at -80°C until analysis.

297 Each fecal sample (~20 mg) was homogenized in 200 µL MeOH:PBS (4:1, v:v, containing
298 1,810.5 µM $^{13}C_3$-lactate and 142 µM $^{13}C_5$-glutamic Acid) in an Eppendorf tube using a Bullet
299 Blender homogenizer (Next Advance, Averill Park, NY). Then 800 µL MeOH:PBS (4:1, v:v,
300 containing 1,810.5 µM $^{13}C_3$-lactate and 142 µM $^{13}C_5$-glutamic Acid) was added, and after
301 vortexing for 10 s, the samples were stored at -20ºC for 30 min. The samples were then sonicated
302 in an ice bath for 30 min. The samples were centrifuged at 14,000 RPM for 10 min (4ºC), and 800
303 µL supernatant was transferred to a new Eppendorf tube. The samples were then dried under
304 vacuum using a CentriVap Concentrator (Labconco, Fort Scott, KS). Prior to MS analysis, the
305 obtained residue was reconstituted in 150 µL 40% PBS/60% ACN. A quality control (QC) sample
306 was pooled from all the study samples.
307

308 **Solutions for Experiments**

309 Solutions used to complete analysis included, acetonitrile (ACN), methanol (MeOH),
310 ammonium acetate, and acetic acid, all LC-MS grade, were purchased from Fisher Scientific
311 (Pittsburgh, PA). Ammonium hydroxide was bought from Sigma-Aldrich (Saint Louis, MO). DI
312 water was provided in-house by a Water Purification System from EMD Millipore (Billerica, MA).
313 PBS was bought from GE Healthcare Life Sciences (Logan, UT). The standard compounds
314 corresponding to the measured metabolites were purchased from Sigma-Aldrich (Saint Louis, MO)
315 and Fisher Scientific (Pittsburgh, PA).

316

317 **Untargeted LC-MS Metabolomics**

318 The untargeted LC-MS metabolomics method used has developed and used in a growing
319 number of studies (Garcia et al., 2024; Gu et al., 2015; D. Scieszka et al., 2023; D. P. Scieszka et
320 al., 2023; Wei et al., 2021). Briefly, all LC-MS experiments were performed using the Thermo
321 Vanquish UPLC-Exploris 240 Orbitrap MS instrument (Waltham, MA). Fecal samples were
322 injected twice, 10 µL for analysis using negative ionization mode and 4µL for analysis using
323 positive ionization mode. Both chromatographic separations were performed in hydrophilic
324 interaction chromatography (HILIC) mode on a Waters XBridge BEH Amide column (150 x 2.1
325 mm, 2.5 µm particle size, Waters Corporation, Milford, MA). The flow rate was 0.3 mL/min, auto-
326 sampler temperature was kept at 4℃, and the column compartment was set at 40℃. The mobile
327 phase was composed of Solvents A (10 mM ammonium acetate, 10 mM ammonium hydroxide in
328 95% H_2O/5% ACN) and B (10 mM ammonium acetate, 10 mM ammonium hydroxide in 95%
329 ACN/5% H_2O). After the initial 1 min isocratic elution of 90% B, the percentage of Solvent B
330 decreased to 40% at t = 11 min. The composition of Solvent B maintained at 40% for 4 min (t=15

331 min), and then the percentage of B gradually went back to 90%, to prepare for the next injection.
332 Using mass spectrometer equipped with an electrospray ionization (ESI) source, we will collect
333 untargeted data from 70 to 1050 m/z.

334 To identify peaks from the MS spectra, we made extensive use of the in-house chemical
335 standards (~600 aqueous metabolites), and in addition, we searched the resulting MS spectra
336 against the HMDB library, Lipidmap database, METLIN database, as well as commercial
337 databases including mzCloud, Metabolika, and ChemSpider. The absolute intensity threshold for
338 the MS data extraction was 1,000, and the mass accuracy limit was set to 5 ppm. Identifications
339 and annotations used available data for retention time (RT), exact mass (MS), MS/MS
340 fragmentation pattern, and isotopic pattern. Thermo Compound Discoverer 3.3 software was used
341 for aqueous metabolomics data processing. The untargeted data were processed by the software
342 for peak picking, alignment, and normalization. To improve rigor, only the signals/peaks with CV
343 < 20% across quality control (QC) pools, and the signals showing up in >80% of all the samples
344 were included for further analysis.

345

346 **Data Preprocessing**

347 Raw data files generated from the mass spectrometer were converted to a matrix of peak
348 intensities, with each row representing a sample and each column representing a metabolite
349 feature. Quality control samples were also included in the matrix for inter-batch normalization.
350 Raw data were normalized to the median of quality control samples.

351

352 **Multivariate Analysis**

353 Principal component analysis (PCA) and orthogonal partial least squares-discriminant
354 analysis (OPLS-DA) were performed to explore the separation of different groups and identify
355 important metabolites contributing to the separation.

356 To investigate metabolite differences between sex and maternal dietary groups, as well as
357 their interactions, two-way ANOVA was conducted using GraphPad Prism 10. Significant main
358 effects of two-way ANOVAs were followed by Tukey's post-hoc test to adjust for multiple
359 comparisons.

360 Statistical analysis was performed using R (version 4.1.1). For each metabolite, normality
361 was assessed using the Shapiro-Wilk test, which is suitable for small to medium sample sizes and
362 provides a good balance between sensitivity and specificity.

363 If the data for a metabolite followed a normal distribution ($p > 0.05$ in the Shapiro-Wilk
364 test), independent t-tests were used to compare the means between groups. If the data did not
365 follow a normal distribution ($p \leq 0.05$ in the Shapiro-Wilk test), non-parametric Wilcoxon rank-
366 sum tests were employed to compare the medians between groups. This test was chosen because
367 it does not assume normality and is robust against outliers. To adjust for multiple testing and
368 control the rate of type I errors, a false discovery rate (FDR) correction was performed using the
369 Benjamini-Hochberg procedure.

370 Linear regression analysis was conducted to assess the correlation between metabolites and
371 stroke outcomes. The regression models included sex, maternal diet, and their interaction terms to
372 determine the influence of these factors on metabolite levels.

373

374 **Pathway and Enrichment Analysis**

Pathway analysis was performed using MetaboAnalyst (version 5.0). The pathway analysis involved the use of the hypergeometric test and pathway topological analysis. The p-values were not Bonferroni corrected because the related hypotheses are not completely independent, but instead share underlying biological mechanisms or functional relationships. Enrichment analysis was performed to identify the over-representation of metabolites in specific pathways using Fisher's exact test.

Visualization

Heatmaps were generated to visualize the clustering of metabolites and samples. Pathway and enrichment analysis results were visualized using bubble plots and bar plots, respectively. The level of significance was set at $p < 0.05$ for all analyses.

Acknowledgements

None

Competing Interests

No competing interest declared

Funding

This research was funded by American Heart Association, grant number 20AIREA35050015 awarded to NMJ

Data statement

All relevant data can be found within the article and supplementary material

Author contrition statement

Conceptualization: N.M.J.; Methodology: H.G., J.C.; Formal analysis: P.J., F.A., N.M.J; Writing-review & editing: F.A., M.T.M, P.J., H.G., N.M.J; Visualization: P.J., F.A., N.M.J; Project administration: N.M.J.; Funding acquisition: N.M.J.

Diversity and inclusion statement

All authors were given opportunities to be involved in the study and writing up of the study.

413 **References**

414 Abato, J. E., Moftah, M., Cron, G. O., Smith, P. D., & Jadavji, N. M. (2020). Methylenetetrahydrofolate
415 reductase deficiency alters cellular response after ischemic stroke in male mice. *Nutritional*
416 *Neuroscience*. https://doi.org/10.1080/1028415X.2020.1769412

417 Aydogan Kirmizi, D., Baser, E., & Doganyigit, Z. (2021). The Activation of Cannabinoid Type-2
418 Receptor with JWH-133 Protects Uterine Ischemia/Reperfusion-Induced Damage.
419 *Pharmacology*, *106*(1–2), 106–113. https://doi.org/10.1159/000511457

420 Bailey, R. L., Dodd, K. W., Gahche, J. J., Dwyer, J. T., McDowell, M. A., Yetley, E. A., Sempos, C. A.,
421 Burt, V. L., Radimer, K. L., & Picciano, M. F. (2010). Total folate and folic acid intake from
422 foods and dietary supplements in the United States: 2003–2006. *The American Journal of*
423 *Clinical Nutrition*, *91*(1), 231–237. https://doi.org/10.3945/ajcn.2009.28427

424 Benjamin, E. J., Virani, S. S., Callaway, C. W., Chang, A. R., Cheng, S., Chiuve, S. E., Cushman, M.,
425 Delling, F. N., Deo, R., de Ferranti, S. D., Ferguson, J. F., Fornage, M., Gillespie, C., Isasi, C. R.,
426 Jiménez, M. C., Jordan, L. C., Judd, S. E., Lackland, D., Lichtman, J. H., … Muntner, P. (2018).
427 Heart Disease and Stroke Statistics—2018 Update: A Report From the American Heart
428 Association. In *Circulation*. https://doi.org/10.1161/CIR.0000000000000558

429 Biagioli, M., Carino, A., Cipriani, S., Francisci, D., Marchianò, S., Scarpelli, P., Sorcini, D., Zampella,
430 A., & Fiorucci, S. (2017). The Bile Acid Receptor GPBAR1 Regulates the M1/M2 Phenotype of
431 Intestinal Macrophages and Activation of GPBAR1 Rescues Mice from Murine Colitis. *Journal*
432 *of Immunology (Baltimore, Md.: 1950)*, *199*(2), 718–733.
433 https://doi.org/10.4049/jimmunol.1700183

434 Caldovic, L., Ah Mew, N., Shi, D., Morizono, H., Yudkoff, M., & Tuchman, M. (2010). N-
435 acetylglutamate synthase: Structure, function and defects. *Molecular Genetics and Metabolism*,
436 *100 Suppl 1*(Suppl 1), S13-19. https://doi.org/10.1016/j.ymgme.2010.02.018

437 Campbell, M. E., Williams, S. J., Veerareddy, S., & Davidge, S. T. (2005). Maternal nutrient restriction
438 reduces carotid artery constriction without increasing nitric oxide synthesis in the late gestation

439 rat fetus. *Pediatric Research*, *58*(5), 840–844.
440 https://doi.org/10.1203/01.PDR.0000181376.83137.ED
441 Carolan-Olah, M., Duarte-Gardea, M., & Lechuga, J. (2015). A critical review: Early life nutrition and
442 prenatal programming for adult disease. *Journal of Clinical Nursing*, *24*(23–24), 3716–3729.
443 https://doi.org/10.1111/jocn.12951
444 Chi, N.-F., Chang, T.-H., Lee, C.-Y., Wu, Y.-W., Shen, T.-A., Chan, L., Chen, Y.-R., Chiou, H.-Y., Hsu,
445 C. Y., & Hu, C.-J. (2021). Untargeted metabolomics predicts the functional outcome of ischemic
446 stroke. *Journal of the Formosan Medical Association*, *120*(1, Part 1), 234–241.
447 https://doi.org/10.1016/j.jfma.2020.04.026
448 Clementson, M., Hurley, L., Coonrod, S., Bennett, C., Marella, P., Pascual, A. S., Pull, K., Wasek, B.,
449 Bottiglieri, T., Malysheva, O., Ca Udill, M. A., & Jadavji, N. M. (2022). *Maternal dietary*
450 *deficiencies in folates or choline during pregnancy and lactation worsen stroke outcome in 3-*
451 *month-old male and female mouse offspring* [Preprint]. Neuroscience.
452 https://doi.org/10.1101/2022.09.28.509960
453 Clementson, M., Hurley, L., Coonrod, S., Bennett, C., Marella, P., Pascual, A. S., Pull, K., Wasek, B.,
454 Bottiglieri, T., Malysheva, O., Caudill, M. A., & Jadavji, N. M. (2023). Maternal dietary
455 deficiencies in folic acid or choline worsen stroke outcomes in adult male and female mouse
456 offspring. *Neural Regeneration Research*, *18*(11), 2443–2448. https://doi.org/10.4103/1673-
457 5374.371375
458 Dam, K., Füchtemeier, M., Farr, T. D., Boehm-Sturm, P., Foddis, M., Dirnagl, U., Malysheva, O.,
459 Caudill, M. A., & Jadavji, N. M. (2017). Increased homocysteine levels impair reference memory
460 and reduce cortical levels of acetylcholine in a mouse model of vascular cognitive impairment.
461 *Behavioural Brain Research*, *321*. https://doi.org/10.1016/j.bbr.2016.12.041
462 Di Meco, A., & Praticò, D. (2019). Early-life exposure to high-fat diet influences brain health in aging
463 mice. *Aging Cell*. https://doi.org/10.1111/acel.13040

464 Durrant, L. M., Khorram, O., Buchholz, J. N., & Pearce, W. J. (2014). Maternal food restriction
465 modulates cerebrovascular structure and contractility in adult rat offspring: Effects of
466 metyrapone. *American Journal of Physiology - Regulatory Integrative and Comparative*
467 *Physiology*, *306*(6). https://doi.org/10.1152/ajpregu.00436.2013
468 Feigin, V. L., Forouzanfar, M. H., Krishnamurthi, R., Mensah, G. A., Connor, M., Bennett, D. A., Moran,
469 A. E., Sacco, R. L., Anderson, L., Truelsen, T., O'Donnell, M., Venketasubramanian, N., Barker-
470 Collo, S., Lawes, C. M. M., Wang, W., Shinohara, Y., Witt, E., Ezzati, M., Naghavi, M., …
471 Global Burden of Diseases, Injuries, and Risk Factors Study 2010 (GBD 2010) and the GBD
472 Stroke Experts Group. (2014). Global and regional burden of stroke during 1990-2010: Findings
473 from the Global Burden of Disease Study 2010. *Lancet (London, England)*, *383*(9913), 245–254.
474 Gaillard, R. (2015). Maternal obesity during pregnancy and cardiovascular development and disease in
475 the offspring. *European Journal of Epidemiology*, *30*(11), 1141–1152.
476 https://doi.org/10.1007/s10654-015-0085-7
477 Garcia, M. M., Romero, A. S., Merkley, S. D., Meyer-Hagen, J. L., Forbes, C., Hayek, E. E., Sciezka, D.
478 P., Templeton, R., Gonzalez-Estrella, J., Jin, Y., Gu, H., Benavidez, A., Hunter, R. P., Lucas, S.,
479 Herbert, G., Kim, K. J., Cui, J. Y., Gullapalli, R. R., In, J. G., … Castillo, E. F. (2024). In Vivo
480 Tissue Distribution of Polystyrene or Mixed Polymer Microspheres and Metabolomic Analysis
481 after Oral Exposure in Mice. *Environmental Health Perspectives*, *132*(4), 47005.
482 https://doi.org/10.1289/EHP13435
483 Gernand, A. D., Schulze, K. J., Stewart, C. P., West, K. P., Christian, P., & Christian, P. (2016).
484 Micronutrient deficiencies in pregnancy worldwide: Health effects and prevention. *Nature*
485 *Reviews. Endocrinology*, *12*(5), 274–289. https://doi.org/10.1038/nrendo.2016.37
486 Gu, H., Zhang, P., Zhu, J., & Raftery, D. (2015). Globally Optimized Targeted Mass Spectrometry:
487 Reliable Metabolomics Analysis with Broad Coverage. *Analytical Chemistry*, *87*(24), 12355–
488 12362. https://doi.org/10.1021/acs.analchem.5b03812

489 Hamner, H. C., Cogswell, M. E., & Johnson, M. A. (2011). Acculturation Factors Are Associated with
490 Folate Intakes among Mexican American Women. *The Journal of Nutrition*, *141*(10), 1889–1897.
491 https://doi.org/10.3945/jn.111.143412
492 Hankey, G. J. (2012). Nutrition and the risk of stroke. *The Lancet Neurology*, *11*(1), 66–81.
493 https://doi.org/10.1016/S1474-4422(11)70265-4
494 Harper, M. S., Amanda Shen, Z., Barnett, J. F., Krsmanovic, L., Myhre, A., & Delaney, B. (2009). N-
495 acetyl-glutamic acid: Evaluation of acute and 28-day repeated dose oral toxicity and genotoxicity.
496 *Food and Chemical Toxicology: An International Journal Published for the British Industrial*
497 *Biological Research Association*, *47*(11), 2723–2729. https://doi.org/10.1016/j.fct.2009.07.036
498 Honda, M., Tint, G. S., Honda, A., Nguyen, L. B., Chen, T. S., & Shefer, S. (1998). 7-Dehydrocholesterol
499 down-regulates cholesterol biosynthesis in cultured Smith-Lemli-Opitz syndrome skin
500 fibroblasts. *Journal of Lipid Research*, *39*(3), 647–657.
501 Huang, S. S., Liu, I.-H., Chen, C.-L., Chang, J.-M., Johnson, F. E., & Huang, J. S. (2017). 7-
502 Dehydrocholesterol (7-DHC), But Not Cholesterol, Causes Suppression of Canonical TGF-β
503 Signaling and Is Likely Involved in the Development of Atherosclerotic Cardiovascular Disease
504 (ASCVD). *Journal of Cellular Biochemistry*, *118*(6), 1387–1400.
505 https://doi.org/10.1002/jcb.25797
506 Hurley, L., Jauhal, J., Ille, S., Pull, K., Malysheva, O. V., & Jadavji, N. M. (2023). Maternal Dietary
507 Deficiencies in Folic Acid and Choline Result in Larger Damage Volume, Reduced Neuro-
508 Degeneration and -Inflammation and Changes in Choline Metabolites after Ischemic Stroke in
509 Middle-Aged Offspring. *Nutrients*, *15*(7), 1556. https://doi.org/10.3390/nu15071556
510 Jadavji, N. M., Deng, L., Malysheva, O. V., Caudill, M. A., & Rozen, R. (2015). MTHFR deficiency or
511 reduced intake of folate or choline in pregnant mice results in impaired short-term memory and
512 increased apoptosis in the hippocampus of wild-type offspring. *Neuroscience*, *300*, 1–9.
513 https://doi.org/10.1016/j.neuroscience.2015.04.067

514 Jadavji, N. M., Emmerson, J., Willmore, W. G., MacFarlane, A. J., & Smith, P. (2017). B-vitamin and
515 choline supplementation increases neuroplasticity and recovery after stroke. *Neurobiology of*
516 *Disease*, *103*, 89–100.
517 Jadavji, N. M., Farr, T., Khalil, A., Boehm-Sturm, P., Foddis, M., Harms, C., Füchtemeier, M., &
518 Dirnagl, U. (2015). Elevated levels of plasma homocysteine, deficiencies in dietary folic acid and
519 uracil-DNA glycosylase impair learning in a mouse model of vascular cognitive impairment.
520 *Behavioural Brain Research*, *283*, 215–226. https://doi.org/10.1016/j.bbr.2015.01.040
521 Jadavji, N. M., Mosnier, H., Kelly, E., Lawrence, K., Cruickshank, S., Stacey, S., McCall, A., Dhatt, S.,
522 Arning, E., Bottiglieri, T., & Smith, P. D. (2019). One-carbon metabolism supplementation
523 improves outcome after stroke in aged male MTHFR-deficient mice. *Neurobiology of Disease*,
524 *132*. https://doi.org/10.1016/j.nbd.2019.104613
525 Jensen, H., Batres-Marquez, S., Carriquiry, A., & Schalinske, K. (2007). Choline in the diets of the US
526 population: NHANES, 2003–2004. *Late Breaking Abstracts*.
527 Kang, X.-C., Chen, T., Zhou, J.-L., Shen, P.-Y., Dai, S.-H., Gao, C.-Q., Zhang, J.-Y., Xiong, X.-Y., &
528 Liu, D.-B. (2021). Phytosterols in hull-less pumpkin seed oil, rich in Δ7-phytosterols, ameliorate
529 benign prostatic hyperplasia by lowing 5α-reductase and regulating balance between cell
530 proliferation and apoptosis in rats. *Food & Nutrition Research*, *65*.
531 https://doi.org/10.29219/fnr.v65.7537
532 Kolb, B., Whishaw, I. Q., & Teskey, G. C. (2016). *An Introduction to Brain and Behavior* (Fifth). Worth
533 Publishers. https://doi.org/10.1017/CBO9780511623806
534 Lee, J.-K., Kim, J.-E., Sivula, M., & Strittmatter, S. M. (2004). Nogo receptor antagonism promotes
535 stroke recovery by enhancing axonal plasticity. *The Journal of Neuroscience : The Official*
536 *Journal of the Society for Neuroscience*, *24*(27), Article 27.
537 https://doi.org/10.1523/JNEUROSCI.1643-04.2004
538 Li, X. S., Wang, Z., Cajka, T., Buffa, J. A., Nemet, I., Hurd, A. G., Gu, X., Skye, S. M., Roberts, A. B.,
539 Wu, Y., Li, L., Shahen, C. J., Wagner, M. A., Hartiala, J. A., Kerby, R. L., Romano, K. A., Han,

Y., Obeid, S., Lüscher, T. F., … Hazen, S. L. (2018). Untargeted metabolomics identifies trimethyllysine, a TMAO-producing nutrient precursor, as a predictor of incident cardiovascular disease risk. *JCI Insight*, *3*(6), e99096, 99096. https://doi.org/10.1172/jci.insight.99096

Lin, C. C., Wu, X. Y., Zhou, Y. L., Shao, B., Niu, X. T., Zhang, W. L., & Lin, Y. S. (2018). Maternal high-fat diet programs cerebrovascular remodeling in adult rat offspring. *Journal of Cerebral Blood Flow and Metabolism*, *38*(11), 1954–1967. https://doi.org/10.1177/0271678X17731956

López-Dyck, E., Andrade-Urzúa, F., Elizalde, A., Ferrer-Villada, T., Dagnino-Acosta, A., Huerta, M., Osuna-Calleros, Z., Rangel-Sandoval, C., & Sánchez-Pastor, E. (2017). ACPA and JWH-133 modulate the vascular tone of superior mesenteric arteries through cannabinoid receptors, BKCa channels, and nitric oxide dependent mechanisms. *Pharmacological Reports: PR*, *69*(6), 1131–1139. https://doi.org/10.1016/j.pharep.2017.06.011

Márk, L., & Paragh, G. (2007). [Change in the cholesterol metabolism associated with the combined inhibition of synthesis and absorption]. *Orvosi Hetilap*, *148*(14), 627–632. https://doi.org/10.1556/OH.2007.28065

Mbs, G. B. Y., Wasek, B., Bottiglieri, T., Malysheva, O., Caudill, M. A., & Jadavji, N. M. (2023). Dietary vitamin B12 deficiency impairs motor function and changes neuronal survival and choline metabolism after ischemic stroke in middle-aged male and female mice. *Nutritional Neuroscience*, 1–10. https://doi.org/10.1080/1028415X.2023.2188639

Mediratta, P. K., Gambhir, M., Sharma, K. K., & Ray, M. (2001). Antinociceptive activity of a neurosteroid tetrahydrodeoxycorticosterone (5alpha-pregnan-3alpha-21-diol-20-one) and its possible mechanism(s) of action. *Indian Journal of Experimental Biology*, *39*(12), 1299–1301.

Mozaffarian, D., Benjamin, E. J., Go, A. S., Arnett, D. K., Blaha, M. J., Cushman, M., Das, S. R., de Ferranti, S., Despres, J. P., Fullerton, H. J., Howard, V. J., Huffman, M. D., Isasi, C. R., Jimenez, M. C., Judd, S. E., Kissela, B. M., Lichtman, J. H., Lisabeth, L. D., Liu, S., … Turner, M. B. (2015). Heart Disease and Stroke Statistics-2016 Update: A Report From the American Heart Association. *Circulation*, *133*(4), e38-360. https://doi.org/10.1161/CIR.0000000000000350

566 Nie, Y., Wang, Z., Chai, G., Xiong, Y., Li, B., Zhang, H., Xin, R., Qian, X., Tang, Z., Wu, J., & Zhao, P.
567 (2019). Dehydrocostus Lactone Suppresses LPS-induced Acute Lung Injury and Macrophage
568 Activation through NF-κB Signaling Pathway Mediated by p38 MAPK and Akt. *Molecules*
569 *(Basel, Switzerland)*, *24*(8), 1510. https://doi.org/10.3390/molecules24081510
570 Onat, T., Demir Caltekin, M., Doğanyigit, Z., Turkler, C., Kaymak, E., Kara, M., & Serdar Yalvac, E.
571 (2021). Activation of cannabinoid 2 receptors by JWH-133 protects against ovarian ischemia-
572 reperfusion injury in rats. *Biotechnic & Histochemistry: Official Publication of the Biological*
573 *Stain Commission*, *96*(5), 394–400. https://doi.org/10.1080/10520295.2021.1905183
574 Ovbiagele, B., Goldstein, L. B., Higashida, R. T., Howard, V. J., Johnston, S. C., Khavjou, O. A.,
575 Lackland, D. T., Lichtman, J. H., Mohl, S., Sacco, R. L., Saver, J. L., & Trogdon, J. G. (2013).
576 Forecasting the future of stroke in the united states: A policy statement from the American heart
577 association and American stroke association. *Stroke*, *44*(8), 2361–2375.
578 https://doi.org/10.1161/STR.0b013e31829734f2
579 Paik, M.-J., Lee, S., Cho, K.-H., & Kim, K.-R. (2006). Urinary polyamines and N-acetylated polyamines
580 in four patients with Alzheimer's disease as their N-ethoxycarbonyl-N-pentafluoropropionyl
581 derivatives by gas chromatography-mass spectrometry in selected ion monitoring mode.
582 *Analytica Chimica Acta*, *576*(1), 55–60. https://doi.org/10.1016/j.aca.2006.01.070
583 Palinski, W. (2014). Effect of maternal cardiovascular conditions and risk factors on offspring
584 cardiovascular disease. *Circulation*, *129*(20), 2066–2077.
585 https://doi.org/10.1161/CIRCULATIONAHA.113.001805
586 Pannia, E., Cho, C. E., Kubant, R., Sánchez-Hernández, D., Huot, P. S. P., & Harvey Anderson, G.
587 (2016). Role of maternal vitamins in programming health and chronic disease. *Nutrition Reviews*,
588 *0*(0), nuv103. https://doi.org/10.1093/nutrit/nuv103
589 Patchev, V. K., Montkowski, A., Rouskova, D., Koranyi, L., Holsboer, F., & Almeida, O. F. (1997).
590 Neonatal treatment of rats with the neuroactive steroid tetrahydrodeoxycorticosterone (THDOC)

591 abolishes the behavioral and neuroendocrine consequences of adverse early life events. *The*
592 *Journal of Clinical Investigation*, *99*(5), 962–966. https://doi.org/10.1172/JCI119261
593 Pedroso, J. A. B., Zampieri, T. T., & Donato, J. (2015). Reviewing the Effects of L-Leucine
594 Supplementation in the Regulation of Food Intake, Energy Balance, and Glucose Homeostasis.
595 *Nutrients*, *7*(5), 3914–3937. https://doi.org/10.3390/nu7053914
596 Piette, P. C. M. (2020). The pharmacodynamics and safety of progesterone. *Best Practice & Research.*
597 *Clinical Obstetrics & Gynaecology*, *69*, 13–29. https://doi.org/10.1016/j.bpobgyn.2020.06.002
598 Poole, J., Jasbi, P., Pascual, A. S., North, S., Kwatra, N., Weissig, V., Gu, H., Bottiglieri, T., & Jadavji,
599 N. M. (2022). *Reduced stroke outcome in old-aged female mice maintained on a dietary vitamin*
600 *B12 deficiency* (p. 2022.04.04.487028). bioRxiv. https://doi.org/10.1101/2022.04.04.487028
601 Prokopová, I. (2010). [Noradrenaline and behavior]. *Ceskoslovenska Fysiologie*, *59*(2), 51–58.
602 Pull, K., Folk, R., Kang, J., Jackson, S., Gusek, B., Esfandiarei, M., & Jadavji, N. M. (2023). Impact of
603 maternal dietary folic acid or choline dietary deficiencies on vascular function in young and
604 middle-aged female mouse offspring after ischemic stroke. *American Journal of Physiology.*
605 *Heart and Circulatory Physiology*, *325*(6), H1354–H1359.
606 https://doi.org/10.1152/ajpheart.00502.2023
607 Razak, M. A., Begum, P. S., Viswanath, B., & Rajagopal, S. (2017). Multifarious Beneficial Effect of
608 Nonessential Amino Acid, Glycine: A Review. *Oxidative Medicine and Cellular Longevity*, *2017*,
609 1716701. https://doi.org/10.1155/2017/1716701
610 Rupprecht, R. (1997). The neuropsychopharmacological potential of neuroactive steroids. *Journal of*
611 *Psychiatric Research*, *31*(3), 297–314. https://doi.org/10.1016/s0022-3956(96)00060-x
612 Saul, R., Chambers, J. P., Molyneux, R. J., & Elbein, A. D. (1983). Castanospermine, a tetrahydroxylated
613 alkaloid that inhibits beta-glucosidase and beta-glucocerebrosidase. *Archives of Biochemistry and*
614 *Biophysics*, *221*(2), 593–597. https://doi.org/10.1016/0003-9861(83)90181-9
615 Scieszka, D., Jin, Y., Noor, S., Barr, E., Garcia, M., Begay, J., Herbert, G., Hunter, R. P., Bhaskar, K.,
616 Kumar, R., Gullapalli, R., Bolt, A., McCormick, M. A., Bleske, B., Gu, H., & Campen, M. J.

617 (2023). Biomass smoke inhalation promotes neuroinflammatory and metabolomic temporal
618 changes in the hippocampus of female mice. *Journal of Neuroinflammation*, *20*, 192.
619 https://doi.org/10.1186/s12974-023-02874-y
620 Scieszka, D. P., Garland, D., Hunter, R., Herbert, G., Lucas, S., Jin, Y., Gu, H., Campen, M. J., &
621 Cannon, J. L. (2023). Multi-omic assessment shows dysregulation of pulmonary and systemic
622 immunity to e-cigarette exposure. *Respiratory Research*, *24*(1), 138.
623 https://doi.org/10.1186/s12931-023-02441-2
624 Shikata, K., Niiro, H., Azuma, H., Ogino, K., & Tachibana, T. (2003). Apoptotic activities of C2-
625 ceramide and C2-dihydroceramide homologues against HL-60 cells. *Bioorganic & Medicinal*
626 *Chemistry*, *11*(13), 2723–2728. https://doi.org/10.1016/s0968-0896(03)00228-1
627 Smajlović, D. (2015). Strokes in young adults: Epidemiology and prevention. *Vascular Health and Risk*
628 *Management*, *11*, 157–164. https://doi.org/10.2147/VHRM.S53203
629 Spence, J. D., Yi, Q., & Hankey, G. J. (2017). B vitamins in stroke prevention: Time to reconsider. *The*
630 *Lancet Neurology*, *16*(9), 750–760. https://doi.org/10.1016/S1474-4422(17)30180-1
631 Sumien, N., Shetty, R. A., & Gonzales, E. B. (2018). Creatine, Creatine Kinase, and Aging. *Sub-Cellular*
632 *Biochemistry*, *90*, 145–168. https://doi.org/10.1007/978-981-13-2835-0_6
633 Tharappel, A. M., Cheng, Y., Holmes, E. H., Ostrander, G. K., & Tang, H. (2020). Castanospermine
634 reduces Zika virus infection-associated seizure by inhibiting both the viral load and inflammation
635 in mouse models. *Antiviral Research*, *183*, 104935.
636 https://doi.org/10.1016/j.antiviral.2020.104935
637 Tong, X., George, M. G., Gillespie, C., & Merritt, R. (2016). Trends in hospitalizations and cost
638 associated with stroke by age, United States 2003–2012. *International Journal of Stroke*, *11*(8),
639 874–881. https://doi.org/10.1177/1747493016654490
640 Waterham, H. R., & Wanders, R. J. (2000). Biochemical and genetic aspects of 7-dehydrocholesterol
641 reductase and Smith-Lemli-Opitz syndrome. *Biochimica Et Biophysica Acta*, *1529*(1–3), 340–
642 356. https://doi.org/10.1016/s1388-1981(00)00159-1

643 Wei, Y., Jasbi, P., Shi, X., Turner, C., Hrovat, J., Liu, L., Rabena, Y., Porter, P., & Gu, H. (2021). Early
644 Breast Cancer Detection Using Untargeted and Targeted Metabolomics. *Journal of Proteome*
645 *Research*, *20*(6), 3124–3133. https://doi.org/10.1021/acs.jproteome.1c00019
646 Wetzel, C. H., Vedder, H., Holsboer, F., Zieglgänsberger, W., & Deisz, R. A. (1999). Bidirectional effects
647 of the neuroactive steroid tetrahydrodeoxycorticosterone on GABA-activated Cl- currents in
648 cultured rat hypothalamic neurons. *British Journal of Pharmacology*, *127*(4), 863–868.
649 https://doi.org/10.1038/sj.bjp.0702597
650 Wojcieszak, J., Krzemień, W., & Zawilska, J. B. (2016). JWH-133, a Selective Cannabinoid CB_2
651 Receptor Agonist, Exerts Toxic Effects on Neuroblastoma SH-SY5Y Cells. *Journal of Molecular*
652 *Neuroscience: MN*, *58*(4), 441–445. https://doi.org/10.1007/s12031-016-0726-7
653 Womack, M. D., Pyner, S., & Barrett-Jolley, R. (2006). Inhibition by alpha-
654 tetrahydrodeoxycorticosterone (THDOC) of pre-sympathetic parvocellular neurones in the
655 paraventricular nucleus of rat hypothalamus. *British Journal of Pharmacology*, *149*(5), 600–607.
656 https://doi.org/10.1038/sj.bjp.0706911
657 Wu, M.-H., Chang, C.-T., Lin, Y.-N., & Chen, C.-J. (2023). Identification of a potential prognostic
658 plasma biomarker of acute ischemic stroke via untargeted LC-MS metabolomics. *PROTEOMICS*
659 *– Clinical Applications*, *17*(6), 2200081. https://doi.org/10.1002/prca.202200081
660 Wu, Q., Ma, Y., Liu, Y., Wang, N., Zhao, X., & Wen, D. (2020). CB2R agonist JWH-133 attenuates
661 chronic inflammation by restraining M1 macrophage polarization via Nrf2/HO-1 pathway in diet-
662 induced obese mice. *Life Sciences*, *260*, 118424. https://doi.org/10.1016/j.lfs.2020.118424
663 Xiao, J., Li, W., Zheng, X., Qi, L., Wang, H., Zhang, C., Wan, X., Zheng, Y., Zhong, R., Zhou, X., Lu,
664 Y., Li, Z., Qiu, Y., Liu, C., Zhang, F., Zhang, Y., Xu, X., Yang, Z., Chen, H., … Wang, H.
665 (2020). Targeting 7-Dehydrocholesterol Reductase Integrates Cholesterol Metabolism and IRF3
666 Activation to Eliminate Infection. *Immunity*, *52*(1), 109-122.e6.
667 https://doi.org/10.1016/j.immuni.2019.11.015

668 Yessoufou, A., & Moutairou, K. (2011). Maternal Diabetes in Pregnancy: Early and Long-Term
669 Outcomes on the Offspring and the Concept of " Metabolic Memory ." *Journal of Diabetes*
670 *Research*, *2011*. https://doi.org/10.1155/2011/218598
671 Zanatta, Â., Rodrigues, M. D. N., Amaral, A. U., Souza, D. G., Quincozes-Santos, A., & Wajner, M.
672 (2016). Ornithine and Homocitrulline Impair Mitochondrial Function, Decrease Antioxidant
673 Defenses and Induce Cell Death in Menadione-Stressed Rat Cortical Astrocytes: Potential
674 Mechanisms of Neurological Dysfunction in HHH Syndrome. *Neurochemical Research*, *41*(9),
675 2190–2198. https://doi.org/10.1007/s11064-016-1933-x
676 Zeisel, S. H. (2013). Nutrition in pregnancy: The argument for including a source of choline.
677 *International Journal of Women's Health*, *5*, 193–199. https://doi.org/10.2147/IJWH.S36610
678 Zhao, L., Wang, C., Peng, S., Zhu, X., Zhang, Z., Zhao, Y., Zhang, J., Zhao, G., Zhang, T., Heng, X., &
679 Zhang, L. (2022). Pivotal interplays between fecal metabolome and gut microbiome reveal
680 functional signatures in cerebral ischemic stroke. *Journal of Translational Medicine*, *20*(1), 459.
681 https://doi.org/10.1186/s12967-022-03669-0
682
683
684
685
686
687
688
689
690
691
692

693 **Table 1**. The impact of maternal dietary deficiencies in folic acid or choline on offspring fecal
694 metabolite levels prior to ischemic stroke and summary of two-way ANOVA results.
695

Metabolite	Sex of mice	CD	FADD	ChDD	*p*-values Interaction Sex Maternal diet
HEPES	Females	5.56 ± 0.04	5.29 ± 0.02	6.41 ± 0.22	0.01
	Males	5.68 ± 0.20	5.39 ± 0.04	5.41 ± 0.03	0.994
					0.00052

696
697
698 Values are expressed as the mean ± SEM of 5 to 7 mice per group. Abbreviations: CD: Control
699 diet; ChDD: choline-deficient diet; FADD: folic acid-deficient diet.
700

701

702 **Table 2.** The impact of maternal dietary deficiencies in folic acid or choline on offspring fecal

703 metabolite levels 1 week post ischemic stroke and summary of two-way ANOVA results.

704

Metabolite	**Sex of mice**	**CD**	**FADD**	**ChDD**	***p*-values Interaction Sex Maternal diet**
PALDA	Female	5.80 ± 0.193	4.32 ± 0.07	5.41 ± 0.25	0.0213
	Male	5.04 ± 0.27	4.65 ± 0.19	4.69 ± 0.14	0.0307
					0.0006
N6-(2-1Himidazol-4-yl)ethyl)lysine	Females	5.05 ± 0.03	4.84 ± 0.03	5.04 ± 0.03	n.s.
	Males	4.97 ± 0.02	4.83 ± 0.02	4.97 ± 0.03	0.0221
					0.0001
3-Oxopalmitic Acid	Female	4.87 ± 0.01	4.79 ± 0.08	4.86 ± 0.04	0.001
	Male	5.56 ± 0.13	5.15 ± 0.11	5.01 ± 0.14	n.s.
					0.017
7-Dehydrocholesterol	Female	6.27 ± 0.04	4.464 ± 0.01	6.087 ± 0.10	0.0001
	Male	5.35 ± 0.19	5.352 ± 0.19	5.459 ± 0.13	n.n.
					0.0001
Yakkasterone	Female	6.05 ± 0.16	4.89 ± 0.26	6.097 ± 0.10	n.s.
	Male	6.21 ± 0.35	5.01 ± 0.13	6.23 ± 0.10	n.s.
					0.0001
Tetrahydrodeoxycorticosterone	Female	6.49 ± 0.38	6.58 ± 0.33	4.68 ± 0.16	0.001
	Male	6.26 ± 0.08	5.53 ± 0.08	6.45 ± 0.06	n.s.
					0.013
Avenasterol	Female	6.36 ± 0.11	4.64 ± 0.04	6.08 ± 0.10	0.011
	Male	5.86 ± 0.28	5.50 ± 0.30	6.17 ± 0.09	n.s.
					n.s.
4betamethylzymosterol-4-carbaldehyde	Female	6.52 ± 0.06	5.01 ± 0.04	6.44 ± 0.12	n.s.
	Male	6.16 ± 0.36	5.69 ± 0.26	6.59 + 0.12	n.s.
					0.001
4a,10-Dihydroxy-2,2,6a,6b,9,9,12a-heptamethyl-2,3,4,4a,6,6a,6b	Female	6.71 ± 0.09	5.12 ± 0.20	6.713 ± 0.13	n.s.
	Male	6.38 ± 0.43	5.88 ± 0.40	6.907 ± 0.07	n.s.
					0.004
3-dehydro-6-deoxoteasterone	Female	6.14 ± 0.16	4.87 ± 0.09	5.83 ± 0.15	n.s.
	Male	5.96 ± 0.21	5.48 ± 0.26	6.01 ± 0.03	n.s.
					0.0001
2-Aminoethyl(2R)-3-[(1Z)-1-h(14Z)-14-Tricosen-10-one	Female	6.18 ± 0.19	4.89 ± 0.11	5.74 ± 0.30	n.s.
	Male	5.71 ± 0.21	5.16 ± 0.13	5.96 ± 0.081	n.s.
					0.0001
1-Piperidinocyclohexanecarbonitrile	Female	6.41 ± 0.18	5.75 ± 0.12	6.69 ± 0.14	n.s.
	Male	6.28 ± 0.15	5.70 ± 0.06	6.52 ± 0.11	n.s.
					0.0001
1-(3,5-Dihydroxyphenyl)-2-heptadecanone	Female	6.13 ± 0.07	4.66 ± 0.07	6.00 ± 0.13	n.s.
	Male	6.23 ± 0.40	4.82 ± 0.07	6.30 ± 0.14	n.s.

					0.0001
(R)-palmiticmonoisopropanolamide	Female	6.46 ± 0.09	3.81 ± 0.03	6.10 ± 0.31	0.05
	Male	5.78 ± 0.48	5.15 ± 0.61	6.49 ± 0.12	n.s.
					0.0002
(22S)-22-hydroxycampest-4-en-3-one	Female	6.81 ± 0.16	4.73 ± 0.02	6.53 ± 0.18	0.0099
	Male	6.23 ± 0.37	6.00 ± 0.41	6.40 ± 0.21	n.s.
					0.006
(14Z)-14-Tricosen-10-one	Female	6.81 ± 0.16	4.84 ± 0.04	6.44 ± 0.39	0.0004
	Male	6.24 ± 0.36	6.58 ± 0.12	6.02 ± 0.21	n.s.
					0.0208
(3S,5E,7E,24R)-(6,19,19-~2~H_3_)-9,10-Secocholesta-5,7,10-trie	Female	5.92 ± 0.09	4.35 ± 0.02	5.98 ± 0.09	n.s.
	Male	5.78 ± 0.32	5.01 ± 0.26	5.93 ± 1.00	n.s.
					0.0001
Metabolite	**Sex of mice**	**CD**	**FADD**	**ChDD**	***p*-values Interaction Sex Maternal diet**
PALDA	Female	5.80 ± 0.193	4.32 ± 0.07	5.41 ± 0.25	0.0213

Values are expressed as the mean ± SEM of 5 to 7 mice per group. Abbreviations: CD: Control diet; ChDD: choline-deficient diet; FADD: folic acid-deficient diet.

746 **Table 3.** The impact of maternal dietary deficiencies in folic acid or choline on offspring fecal
747 metabolite levels 4 weeks post ischemic stroke and summary of two-way ANOVA results.

Metabolite	**Sex of mice**	**CD**	**FADD**	**ChDD**	***p*-values Interaction Sex Maternal diet**
HEPES	Females	5.27 ± 0.03	5.41 ± 0.02	5.50 ± 0.06	0.032
	Males	5.39 ± 0.03	5.44 ± 0.04	5.42 ± 0.03	n.s.
					0.0072
Fenamole	Female	5.89 + 0.14	6.52 + 0.067	6.12 + 0.085	n.s.
	Male	6.46 ± 0.12	5.92 ± 0.058	6.37 ± 0.042	0.03
					0.02
2,3-Dihydroxypropylstearate	Female	6.43 ± 0.21	6.41 ± 0.12	6.32 ± 0.21	n.s.
	Male	6.24 ± 0.15	6.13 ± 0.07	6.27 ± 0.11	n.s.
					0.0004
n-Hexanamide	Female	5.89 ± 0.29	6.69 ± 0.04	6.67 ± 0.02	0.0073
	Male	7.25 ± 0.13	7.25 ± 0.35	6.57 ± 0.30	0.0019
					n.s.
N-Acetyl-Lglutamic Acid	Female	7.01 ± 0.15	7.27 ± 0.12	7.24 ± 0.10	0.0029
	Male	7.75 ± 0.11	7.18 ± 0.11	7.20 ± 0.13	0.051
					n.s.
JWH 133	Female	6.48 ± 0.29	6.68 ± 0.08	6.75 ± 0.07	n.s.
	Male	6.63 ± 0.06	6.68 ± 0.07	6.74 ± 0.05	n.s.
					<0.0001
Homocitrulline	Female	5.68 ± 0.03	6.27 ± 0.09	5.91 ± 0.07	0.0001
	Male	6.32 ± 0.08	5.72 ± 0.12	5.94 ± 0.16	n.s.
					n.s.
Sphinganine	Female	6.73 ± 0.17	7.14 ± 0.03	7.07 ± 0.03	0.018
	Male	7.16 ± 0.03	7.11 ± 0.03	7.07 ± 0.01	0.02

					0.053
R-(+)-Etiracetam	Female	5.21 ± 0.22	5.99 + 0.04	5.71 ± 0.05	0.0005
	Male	5.81 ± 0.06	5.60 ± 0.10	5.57 ± 0.09	n.s.
					n.s.
Pyrrolidine	Female	5.59 ± 0.10	6.08 ± 0.12	5.75 ± 0.12	0.01
	Male	6.14 ± 0.09	5.91 ± 0.12	5.93 ± 0.10	0.04
					n.s.
1-(4-Aminobutyl)urea	Female	4.61 ± 0.19	5.94 ± 0.08	5.44 ± 0.17	0.0015
	Male	5.40 ± 0.09	5.15 ± 0.34	5.30 ± 0.24	n.s.
					0.025
Propoxur	Female	5.31 ± 0.19	6.017 ± 0.07	5.93 ± 0.07	n.s.
	Male	5.94 ± 0.14	6.072 ± 0.09	6.18 ± 0.14	0.006
					0.003
PRIMA-1	Female	5.20 ± 0.22	6.00 ± 0.04	5.71 ± 0.05	0.0005
	Male	5.81 ± 0.06	5.60 ± 0.10	5.57 ± 0.09	n.s.
					n.s.
Phytosphingosine	Female	7.56 ± 0.21	8.14 ± 0.19	8.02 ± 0.07	0.022
	Male	8.13 ± 0.07	8.13 ± 0.018	8.15 ± 0.02	0.012
					0.02
N-lauroylglycine	Female	5.96 ± 0.13	6.46 ± 0.04	6.50 ± 0.09	0.0036
	Male	6.34 ± 0.09	6.22 ± 0.11	6.32 ± 0.04	n.s.
					0.021
Nicaraven	Female	5.24 ± 0.09	4.88 ± 0.07	4.84 ± 0.05	n.s.
	Male	5.06 ± 0.07	5.02 ± 0.10	4.94 ± 0.03	n.s.
					0.003

N~6~,N~6~-Dimethyllysine	Female	5.05 ± 0.14	5.78 ± 0.07	5.47 ± 0.13	0.0088
	Male	5.45 ± 0.12	5.31 ± 0.16	5.63 ± 0.18	n.s.
					0.049
Misoprostol	Female	6.18 ± 0.14	5.85 ± 0.08	5.69 ± 0.05	0.032
	Male	5.88 ± 0.05	5.82 ± 0.11	5.91 ± 0.10	n.s.
					0.036
Lovastatin	Female	6.86 ± 0.13	6.43 ± 0.10	6.52 ± 0.05	n.s.
	Male	6.61 ± 0.04	6.50 ± 0.05	6.53 ± 0.08	n.s.
					0.0081
HMMNI	Female	4.87 ± 0.19	5.48 ± 0.23	5.50 ± 0.05	n.s.
	Male	6.08 ± 0.19	6.03 ± 0.19	5.78 ± 0.44	0.0014
					n.s.
Gly-Leu	Female	5.38 ± 0.10	6.46 ± 0.13	5.96 ± 0.11	0.003
	Male	6.21 ± 0.15	6.07 ± 0.30	5.88 ± 0.20	n.s.
					0.026
Chamazulene	Female	5.78 ± 0.35	5.01 ± 0.01	4.929 ± 0.02	0.02
	Male	4.99 ± 0.03	4.97 ± 0.03	4.99 ± 0.02	0.05
					0.02
Esmolol	Female	6.00 ± 0.22	6.39 ± 0.07	6.43 ± 0.04	0.013
	Male	6.59 ± 0.03	6.51 ± 0.08	6.35 ± 0.06	0.025
					0.0364
Dodecylethanolamide	Female	6.38 ± 0.09	6.15 ± 0.03	6.16 ± 0.01	0.038
	Male	6.24 ± 0.02	6.23 ± 0.03	6.20 ± 0.02	n.s.
					0.009
Creatine	Female	7.21 ± 0.17	6.86 ± 0.20	7.06 ± 0.14	0.01
	Male	6.21 ± 0.22	6.98 ± 0.10	7.15 ± 0.33	n.s.

					n.s.
C2 Dihydroceramide	Female	5.55 ± 0.09	5.87 ± 0.06	5.88 ± 0.03	0.003
	Male	5.93 ± 0.04	5.73 ± 0.13	5.84 ± 0.05	n.s.
					n.s.
BAR501	Female	4.61 ± 0.06	5.15 ± 0.04	4.96 ± 0.09	0.006
	Male	5.06 ± 0.08	4.99 ± 0.11	4.97 ± 0.15	n.s.
					0.042
APM	Female	5.59 ± 0.26	6.42 ± 0.07	6.21 ± 0.10	0.024
	Male	6.16 ± 0.12	6.13 ± 0.14	6.14 ± 0.13	n.s.
					0.038
Adenine	Female	6.51 ± 0.14	7.04 ± 0.20	6.91 ± 0.21	0.012
	Male	7.39 ± 0.16	6.76 ± 0.19	7.00 ± 0.15	n.s.
					n.s.
Acetylcadaverine	Female	5.31 ± 0.01	5.80 ± 0.16	5.42 ± 0.05	0.0038
	Male	5.58 ± 0.07	5.44 ± 0.06	5.38 ± 0.03	n.s.
					0.041
Acetohydroxamic acid	Female	7.31 ± 0.08	7.75 ± 0.07	7.48 ± 0.09	0.0084
	Male	7.83 ± 0.12	7.53 ± 0.11	7.73 ± 0.17	n.s.
					n.s.
5-Hydroxytryptophol	Female	5.32 ± 0.14	6.02 ± 0.07	5.93 ± 0.07	n.s.
	Male	5.94 ± 0.19	6.07 ± 0.09	6.18 ± 0.14	0.006
					0.003
1-PP	Female	5.61 ± 0.08	6.07 ± 0.08	5.79 ± 0.10	0.011
	Male	6.14 ± 0.13	5.85 ± 0.12	6.01 ± 0.16	n.s.
					n.s.
(4R)-5-Hydroxy-L-leucine	Female	5.91 ± 0.16	6.66 ± 0.10	6.20 ± 0.13	0.003

	Male	6.64 ± 0.13	6.25 ± 0.25	6.37 ± 0.08	n.s.
					n.s.
(+)-castanospermine	Female	5.05 ± 0.29	5.94 ± 0.08	5.59 ± 0.10	0.028
	Male	5.60 ± 0.08	5.59 ± 0.10	5.69 ± 0.14	n.s.
					0.026

Values are expressed as the mean ± SEM of 5 to 7 mice per group. Abbreviations: CD: Control diet; ChDD: choline-deficient diet; FADD: folic acid-deficient diet.

766 **Table 4**. One-week post-stroke Tukey's pairwise comparison summary determining the impact of
767 maternal dietary deficiencies in folic acid or choline on offspring fecal metabolite levels.
768

	Female				Male		
Metabolite	**CD vs. FADD**	**CD vs. ChDD**	**ChDD vs. FADD**		**CD vs. FADD**	**CD vs. ChDD**	**ChDD vs. FADD**
PALDA	0.002	n.s.	0.0045		n.s.	n.s.	n.s.
N6-(2-1Himidazol-4-yl)ethyl)lysine	0.0002	n.s.	0.0003		0.0044	n.s.	0.0002
3-Oxopalmitic Acid	n.s.	n.s.	n.s.		< 0.001	< 0.001	n.s.
7-Dehydrocholesterol	< 0.001	< 0.001	n.s.		n.s.	n.s.	n.s.
Yakkasterone	0.0013	n.s.	0.001		0.003	n.s.	0.0001
Tetrahydrodeoxycorticosterone	n.s.	0.0002	0.0001		n.s.	n.s.	0.0276
Avenasterol	0.001	n.s.	0.0003		n.s.	n.s.	n.s.
4betamethylzymosterol-4-carbaldehyde	0.0004	n.s.	0.0007		n.s.	n.s.	0.0004
4a,10-Dihydroxy-2,2,6a,6b,9,9,12a-heptamethyl-2,3,4,4a,6,6a,6b	0.0034	n.s.	0.003		n.s.	n.s.	0.0354
3-dehydro-6-deoxoteasterone	0.0003	n.s.	0.0043		n.s.	n.s.	n.s.
2-Aminoethyl(2R)-3-[(1Z)-1-h(14Z)-14-Tricosen-10-one	0.0008	n.s.	0.0016		n.s.	n.s.	0.0306
1-Piperidinocyclohexanecarbonitrile	0.0085	n.s.	0.0002		0.0003	n.s.	0
1-(3,5-Dihydroxyphenyl)-2-heptadecanone	0.003	n.s.	0.0001		0.0009	n.s.	n.s.
(R)-palmiticmonoisopropanolamide	0.004	n.s.	0.0004		n.s.	n.s.	0.0017
(22S)-22-hydroxycampest-4-en-3-one	0.0002	n.s.	0.0006		n.s.	n.s.	n.s.
(14Z)-14-Tricosen-10-one)	0.0002	n.s.	0.0018		n.s.	n.s.	n.s.
(3S,5E,7E,24R)-(6,19,19-~2~H_3_)-9,10-Secocholesta-5,7,10-trie	0.0255	n.s.	0.007		0.0001	n.s.	< 0.001

769
770 Abbreviations: CD: Control diet; ChDD: choline-deficient diet; FADD: folic acid-deficient diet.
771
772
773
774
775

776

777

778 **Table 5.** Four-week post-stroke Tukey's pairwise comparison summary determining the impact of

779 maternal dietary deficiencies in folic acid or choline on offspring fecal metabolite levels.

780

	Female				Male		
Metabolite	**CD vs. FADD**	**CD vs. ChDD**	**ChDD vs. FADD**		**CD vs. FADD**	**CD vs. ChDD**	**ChDD vs. FADD**
2,3-Dihydroxypropylstearate	n.s.	n.s.	0.0062		0.0163	n.s.	0.0115
n-Hexanamide	0.0277	0.0307	n.s.		n.s.	n.s.	n.s.
N-Acetyl-Lglutamic Acid	n.s.	n.s.	n.s.		0.0096	0.0127	n.s.
JWH 133	n.s.	n.s.	n.s.		n.s.	n.s.	n.s.
Homocitrulline	0.0004	n.s.	0.0265		0.0008	0.0349	
Sphinganine	0.0024	0.0111	n.s.		n.s.	n.s.	n.s.
R-(+)-Etiracetam	0.0001	0.0089	n.s.		n.s.	n.s.	n.s.
Pyrrolidine	0.0094	n.s.	n.s.		n.s.	n.s.	n.s.
1-(4-Aminobutyl)urea	0.0001	0.0114	n.s.		n.s.	n.s.	n.s.
Propoxur	0.0012	0.0042	n.s.		n.s.	n.s.	n.s.
PRIMA-1	0.0001	0.0088	n.s.		n.s.	n.s.	n.s.
Phytosphingosine	0.0012	0.0083	n.s.		n.s.	n.s.	n.s.

N-lauroylglycine	0.0014	0.0007	n.s.		n.s.	n.s.	n.s.
Nicaraven	0.0029	0.0011	n.s.		n.s.	n.s.	n.s.
N~6~,N~6~-Dimethyllysine	0.0014	n.s.	n.s.		n.s.	n.s.	n.s.
Misoprostol	0.0424	0.0019	n.s.		n.s.	n.s.	n.s.
Lovastatin	0.003	0.0203	n.s.		n.s.	n.s.	n.s.
HMMNI	n.s.	n.s.	n.s.		n.s.	n.s.	n.s.
Gly-Leu	0.0003	0.0448	n.s.		n.s.	n.s.	n.s.
Chamazulene	0.0037	0.0015	n.s.		n.s.	n.s.	n.s.
Esmolol	0.0355	0.0204	n.s.		n.s.	n.s.	n.s.
Dodecylethanolamide	0.0018	0.0009	n.s.		n.s.	n.s.	n.s.
Creatine	n.s.	n.s.	n.s.		0.0355	0.0091	n.s.
C2 Dihydroceramide	0.009	0.0062	n.s.		n.s.	n.s.	n.s.
BAR501	0.0005	0.0225	n.s.		n.s.	n.s.	n.s.
APM	0.0017	0.018	n.s.		n.s.	n.s.	n.s.
Adenine	n.s.	n.s.	n.s.		n.s.	n.s.	n.s.
Acetylcadaverine	0.0008	n.s.	0.007		n.s.	n.s.	n.s.
Acetohydroxamic acid	0.0176	n.s.	n.s.		n.s.	n.s.	n.s.
5-Hydroxytryptophol	0.0034	n.s.	n.s.		n.s.	n.s.	n.s.
1-PP	0.0181	n.s.	n.s.		n.s.	n.s.	n.s.
(4R)-5-Hydroxy-L-leucine	0.0034	n.s.	n.s.		n.s.	n.s.	n.s.
(+)-castanospermine	0.0011	0.0443	n.s.		n.s.	n.s.	n.s.
Fenamole	0.0003	n.s.	0.0174		0.0032	n.s.	0.0207

781

782 Abbreviations: CD: Control diet; ChDD: choline-deficient diet; FADD: folic acid-deficient diet.
783

784

785