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3 **Negative calorie foods: An empirical examination of what is fact or fiction**

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5 Running title: Negative calorie foods; fact or fiction

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20 Key words: Negative-calorie foods, nutrition, specific dynamic action, assimilation efficiency, energy
21 budget

22

23 **Summary**

24 This empirical study refutes the existence of negative-calorie foods; however such foods will contribute to a
25 negative energy balance, and thus the loss of body mass.

26

27 **Abstract**

28 A proposed weight loss strategy is to include in one's diet foods that are deemed "negative calorie". In
29 theory, negative-calorie foods are foods for which more energy is expended in their digestion and
30 assimilation than is consumed, thereby resulting in an energy deficit. Commonly listed negative calorie
31 foods are characterized by a high water and fiber content and little fat. Although the existence of negative
32 calorie foods has been largely argued against, no empirical study has fully addressed the validity of foods
33 being negative calorie. We conducted such a study using the omnivorous lizard the bearded dragon (*Pogona*
34 *vitticeps*) and celery as the tested food. Celery tops many lists of negative calorie foods due to its high fiber
35 and low caloric content. Following their consumption of celery meals equaling in mass to 5% of their body
36 mass, we measured from each lizard their postprandial metabolic rates to calculate specific dynamic action
37 (SDA). Feces and urate were collected after meals to determine the energy lost to excretion. The specific
38 energy of the celery meals, feces, and urate was determined by bomb calorimetry. Lizards lost on average
39 29% and 14% of meal energy to feces and urate, respectively, and an additional 33% to SDA, leaving a net
40 gain of 24% of the meal's energy. When considering that only a portion of fecal energy stems from the
41 celery meal, the net gain is expectedly higher. Although this study debunks the validity of celery and other
42 proposed foods as negative calorie, these foods will contribute to generating a negative energy budget and
43 thus the loss of body weight.

44

45 **Introduction**

46 From all forms of media outlets, the public is continuously bombarded by a multitude of dieting and
47 weight loss schemes. One such dieting fad that has populated the internet and social media is a diet that
48 consists of food considered to result in “negative calories”. In theory, these are foods for which more
49 energy is expended in their digestion, assimilation, and nutrient storage than is gained [1-4]. Therefore their
50 consumption results in a caloric deficit due to both the lack of net energy gained and that stored energy (i.e.,
51 fat) must therefore be utilized to fuel the completion of digestion and processing. Negative-calorie foods are
52 generally characterized by a high fiber and water content and low caloric density [3,5,6]. Topping the well-
53 touted lists of negative-calorie foods are celery, lettuce, grapefruit, cucumber, and broccoli [5-8].

54 While the conception of negative-calorie foods may be decades old, its inclusion in diets as a weight
55 lost strategy has gained considerable interests over the past decade (largely via on-line blogs) that have
56 promoted such foods for dieting, improved nutrition, and better health [6,7,9]. Such diet plans have been
57 further popularized by recent dieting books including *Foods That Cause You To Lose Weight* [10] and *The*
58 *Negative Calorie Diet* [11]. The proponents of negative-calorie foods cite that in addition to generating
59 caloric deficits, such foods possess the added benefits of boosting metabolism, controlling appetite,
60 improving glycemic control, and cleansing your colon and liver [7,10]. Hence, the consumption of negative
61 calorie foods results in a “win-win situation” given the multiple benefits to your health and improved weight
62 control [6].

63 However, as soon as it was promoted, the validity that foods exist for which more energy is
64 expended in their consumption than is gained was questioned. Nutritionists and physicians raised doubts of
65 the existence of such foods citing that the cost of meal digestion and assimilation is equivalent to only 5-
66 15% of the energy of the meal [3,12,13]. This cost refers to the accumulated energy expended on gastric
67 acid production, gut peristalsis, enzyme synthesis and secretion, and nutrient absorption and assimilation.
68 For humans this cost is generally referred to as diet-induced thermogenesis (DIT) whereas for other
69 vertebrates and invertebrate it is termed specific dynamic action (SDA), the label and acronym that we will
70 refer to in this report [14]. Therefore when accounting for SDA, it thus assumed that nearly 80-95% of the
71 meal’s energy is still available regardless of meal type. The argument is therefore raised that even though
72 such foods are low in caloric content, there is still a net gain in energy.

73 The few studies that have attempted to test the proposal that certain foods are negative-calorie have
74 focused solely on SDA, and have produced mixed results. A study on a single participant consuming raw

75 and liquefied celery over a 12-hr period found that the subject's metabolic expenditure while eating either of
76 the celery meals exceeded the energy content of the meals [15]. In contrast, Clegg and Cooper [2]
77 calculated that the SDA of 15 female subjects following the consumption of 100 g of raw celery was less
78 than that of the celery's energy. The differences in these findings undoubtedly stem from the inclusion of
79 resting metabolic rate (RMR) in the calculated energy expended in the former study and its exclusion to
80 calculate SDA in the latter study.

81 In light of these studies, it has been frequently acknowledged that there is an absence of any
82 empirical studies that have accurately tested the theory and existence of negative-calorie foods [8,12,13,16].
83 It should also be noted that these previous studies failed to account for the additional loss of energy in feces
84 and urine. Given its relatively high fiber content, celery may inherently be characterized by relatively low
85 digestive and assimilation efficiencies [17,18]. Hence, when combining the energy that is lost to DIT and to
86 feces and urine, is it becomes theoretically more plausible for the consumption of celery to tip the balance in
87 favor of an energy deficit [19].

88 We set out to address this point by employing an empirical approach that will either lend support or
89 refute the claim that celery is a negative-calorie food. We did so by using bearded dragon (*Pogona vitticeps*),
90 an omnivorous lizard native to Australia. Although far removed from humans evolutionarily, bearded
91 dragons share with humans an omnivorous diet and identical sets of mechanisms used to digest, absorb, and
92 assimilate food [20-22]. We quantified for these lizards the energy of their celery meals, the energy
93 expended digesting those meals, and the energy lost in feces and urate. By evaluating these energy tradeoffs,
94 we determined bearded dragons to experience a net gain in energy from their celery meals. However, this
95 gain is rapidly abolished by the lizard's resting metabolism.

96

97 **Materials and methods**

98 **Bearded dragons and their maintenance**

99 The bearded dragon, *Pogona vitticeps* (Ahl), is a medium-sized lizard that inhabits arid desert to
100 semi-arid woodland regions of central Australia [21,23]. It possesses a broad triangular head, rows of spiny
101 scales along its body, and when threatened or during social display will flatten its body with males
102 expanding their darkened throat pouch (thus their name) during social interactions [24]. Bearded dragons
103 are naturally omnivorous and feed opportunistically on leaves, flowers, fruits, invertebrates, and small
104 lizards [21-23]. Due to their docile nature, varied diet, and ease of captive maintenance and reproduction,

105 the bearded dragon has become an extremely popular reptile pet [25,26]. The bearded dragons used in this
106 study were hatched and raised in a laboratory-based colony at the University of Alabama. Lizards were
107 housed individually or in pairs in 76-L aquariums with sand substrate, several rocks for basking, and a water
108 dish with water available ad libitum. Light was provided by fluorescent and UVA/UVB bulbs set on a
109 12L:12D cycle. Room temperature was maintained at 26-29°C and humidity at 50–60%. Lizards were
110 raised on a variety of greens (e.g., kale, collard, mustard), vegetables (e.g., carrots and squash), and
111 calcium/vitamin dusted crickets, mealworms, and cockroaches. The nine lizards used in this study were 4-6
112 years of age and weighed 190.1 – 234.1 g (mean \pm SE = 217.9 \pm 4.9 g) at the beginning of the study.
113 Animal care and experimentation were conducted under an approved protocol (#14-06-0077) from the
114 University of Alabama Institutional Animal Care and Use Committee. All efforts were made to minimize
115 any discomfort to the bearded dragons during experimental procedures.

116

117 **Experimental procedure**

118 We selected celery for this study because it tops many lists of reported negative-calorie foods and
119 bearded dragons will voluntarily eat celery. We standardize meal size to 5% of lizard body mass because it
120 is a meal size easily consumed by bearded dragons and it will generate a significant postprandial metabolic
121 response [14,27]. Prior to metabolic and feeding trials, lizards were fasted for a minimum of 10 days to
122 ensure that they were postabsorptive. For our colony of bearded dragons, we have observed lizards to start
123 passing feces and urate (whitish pellet composed of uric acid) within 2-4 days after feeding. Celery was
124 purchased at a local supermarket (Publix) and used within 24 hours of purchase. To test the claim that celery
125 is a negative-calorie food, we quantified the gross energy of celery meals and compared that to the energy
126 expended on celery digestion and assimilation (SDA) and the energy lost to feces and urate.

127

128 **Determination of SMR, postprandial metabolic response, and SDA**

129 We used closed-system respirometry to quantify for each lizard their standard metabolic rate (SMR)
130 and postprandial metabolic response [28,29]. Fasted lizards were weighed and placed into individual
131 respirometry chambers (2.5-3 L) that were fitted with incurrent and excurrent air ports, each connected to a
132 three-way stopcock. Respirometry chambers were placed into an environmental chamber (model DS54SD;
133 Powers Scientific, Pipersville, Pennsylvania, USA) maintained at 30°C, with ambient air constantly pumped
134 through the respirometry chambers. For each metabolic measurement, an initial 45-mL air sample was

135 pulled from the excurrent port and both incurrent and excurrent ports were closed. An hour later, the
136 excurrent port was opened and a second 45-mL sample was drawn. Air samples were pumped (75 mL min^{-1})
137 through a column of Drierite and CO_2 absorbent (Ascarite) into an O_2 analyzer (S-3A/II, AEI Technologies).
138 We calculated whole animal ($\text{mL}\cdot\text{h}^{-1}$) rates of oxygen consumption ($\dot{V}\text{O}_2$) corrected for standard pressure
139 and temperature using a modification of eq. 9 of Vleck [30].

140 We determined each lizard's SMR from $\dot{V}\text{O}_2$ measurements while fasted. For SMR trials, $\dot{V}\text{O}_2$ was
141 measured in the morning (~ 0700) and evening (~ 1900) for four consecutive days. We assigned for each
142 lizard its SMR as the mean of its two lowest measured $\dot{V}\text{O}_2$ [31]. Following SMR trials, lizards were
143 returned to their cages and fed their pre-weighed celery meals. Once they had completed their meals, lizards
144 were placed back into their respirometry chambers and measurements of $\dot{V}\text{O}_2$ were resumed and continued
145 at 6-h intervals for 2.5 days and thereafter at 12-h intervals for the following 2.5 days. From the
146 postprandial measurements we determined the time span that $\dot{V}\text{O}_2$ was significantly greater than SMR [14].
147 We calculated for each lizard their SDA (kJ) by summing the extra O_2 consumed (mL) above SMR during
148 this time span and multiplying that total by 20.9 J. We assumed for this study that 20.9 J is expended per mL
149 of O_2 consumed given that the nutrient dry matter of the celery is approximately 15% protein, 5% fat, and
150 80% carbohydrate, and generates a respiratory quotient of 0.95 [18,32].

151

152 **Energy content of food, feces, and urate**

153 Energy content of the celery used for each feeding trial and of the feces and urate generated from
154 each efficiency trial was determined by bomb calorimetry. For each metabolic and efficiency trial, five sets
155 of diced celery were dried to a constant mass at 55°C . Once dried, each sample set was reweighed (dry
156 mass) and ground to a homogenous fine powder. A subsample of the powder from each set was placed into
157 pre-weighed gelatin capsule (size 00, Parr Instruments, Moline, Illinois, USA), reweighed, and the capsule
158 and powdered celery were ignited in a bomb calorimeter (model 1266; Parr Instruments, Moline, Illinois,
159 USA) to determine total energy content. We subtracted capsule energy ($19.48 \text{ kJ g}^{-1} * \text{capsule mass}$) from
160 total energy to determine celery energy (kJ g^{-1} dry mass). Specific wet mass energy content of the celery (kJ
161 g^{-1}) was determined as the product of dry mass energy content and the celery's dry mass percentage. The
162 energy content of each ingested meal was calculated as the product of meal mass and wet mass energy
163 content [29]. Over the course of this study, five different batches of freshly-purchased celery were used for
164 SDA and digestive efficiency trials. For each trial, five subsets of diced celery were dried and bombed.

165 Among samples, relative wet and dry masses were quite consistent, averaging $94.7 \pm 0.4\%$ and $5.3 \pm 0.4\%$,
166 respectively. Wet mass energy content among the samples, ranged from 0.615 to 0.933 kJ g^{-1} , averaging
167 $0.722 \pm 0.057 \text{ kJ g}^{-1}$.

168 Feces and urate were collected from lizards housed individually in 76-L glass aquariums lined with
169 laboratory countertop paper (VWR, Radnor, PA, USA) with the non-absorbent side facing upward. Prior to
170 feeding, we emptied their large intestine by gavaging with water which removed residual feces and urate.
171 Once fed, lizards were placed in their respective aquarium and checked twice a day for any deposited feces
172 or urate. Any feces and/or urate found were removed, placed in individual drying trays, and dried to a
173 constant mass at 55°C . After one week, their large intestine was gavaged of any residual feces and urate,
174 which was also dried. For each lizard, we combined separately their feces and urate collected over the one-
175 week period. Feces and urate resulting from these trials were dried, weighed, and bombed in gelatin
176 capsules. After subtracting capsule energy, we calculated for each lizard the total energy of their feces and
177 urate.

178

179 **Statistical analyses**

180 We employed a repeated-measures analysis of variance (ANOVA) to demonstrate the statistical
181 effects of sampling time (pre- and post-feeding) on metabolic rates. In conjunction with the ANOVA, we
182 undertook pairwise mean comparisons (Tukey) in order to identify the post-feeding time point that lizard
183 metabolic rates returned to values that did not differ significantly from prefeeding rates, therefore
184 determining the duration of significantly elevated postprandial metabolism. We report means as mean \pm 1SE.

185

186 **Results**

187 **The cost of meal digestion**

188 Fasted bearded dragons housed in darkened respirometry chambers at 30°C experienced a standard
189 metabolic rate (SMR) that averaged $6.68 \pm 0.33 \text{ mL O}_2 \text{ h}^{-1}$ ($0.030 \pm 0.02 \text{ mL O}_2 \text{ g}^{-1} \text{ h}^{-1}$) (Table 1). Feeding
190 induced a significant increase ($P < 0.0001$) in metabolic rate that peaked for these lizards at 12 – 24 hours
191 postfeeding, at values that averaged $62 \pm 7\%$ greater than SMR (Fig. 1, Table 1). Lizards maintained
192 significantly elevated rates of metabolism for up to 3 days postfeeding. Calculated over this duration, lizards
193 expended on average $2.64 \pm 0.22 \text{ kJ}$ digesting and assimilating their celery meals. This expenditure was
194 equivalent to $33.1 \pm 2.4\%$ of the energy of the ingested celery (i.e., SDA coefficient [14]) (Table 1).

195

196 **Energy lost to feces and urate**

197 Feces and urate began to appear in the cages within 2 days after feeding. Over the one-week period,
198 lizards on average produced 0.14 ± 0.02 g dry of feces and 0.10 ± 0.01 g dry of urate (Table 2). Bomb
199 calorimetry determined that the dry mass-specific energy content of feces and urate averaged 16.8 ± 0.6 and
200 11.1 ± 0.4 kJ g⁻¹, respectively (Table 2). Total energy of feces and urate produced from the single celery
201 meal averaged 2.29 ± 0.33 and 1.06 ± 0.13 kJ, respectively (Table 2).

202

203 **Net energy retained**

204 The celery meals provided lizards with 7.83 ± 0.23 kJ of energy, of which 2.29 kJ was lost as feces,
205 1.06 kJ was excreted as urate, and 2.53 kJ was expended on meal digestion and assimilation, (Table 3).
206 Therefore, the net gain in energy from these meals averaged 1.89 ± 0.17 kJ, which was equivalent to $23.4 \pm$
207 2.1% of the ingested energy. In this study, lizards retained on average nearly a quarter of their meal's energy.

208

209 **Discussion**

210 We set out to test the claim that certain foods exist for which the energy invested in their digestion
211 and absorption, and lost through excretion exceeds the amount of assimilated energy that is gained. Thus,
212 such foods result in a net loss of energy from the body, and therefore have been coined “negative-calorie
213 foods”. Negative-calorie foods are characteristically high in fiber and low in energy due to their low fat and
214 high water content [3,5,6]. We chose celery for this study because it possesses all of these characteristics
215 and is the most cited example of a negative-calorie food [5-8]. Bearded dragons were selected for study
216 because they are naturally omnivorous, possess a GI tract similar to that of omnivorous mammals (including
217 humans), and can easily be studied in the lab due to their docile temperament and willingness to consume
218 celery [20,21,26]. We partitioned the energy of the celery meals into that which is lost in SDA, feces, and
219 urate to determine whether there was any remaining assimilated energy thus gained by the lizards. Although,
220 the celery meals were inherently low in energy, the lizards of this study did achieve a net gain of energy
221 from this meal.

222 On its face value, this empirical study debunks the claim that celery is a “negative-calorie” food, and
223 raises doubts to the proposal that such foods do exist. However, it can be asked whether the cost of celery
224 digestion and energy lost via excretion for the lizards approximates the equivalent cost and loss for humans.

225 Second, if not celery, is there a food that potentially would result in a net loss of energy if consumed? And
226 third, which is central to its proposal for weight loss, can negative-calorie foods generate in practice a
227 negative energy budget? In the following we will address these questions in turn.

228

229 **Costs and efficiencies of meal digestion and assimilation**

230 The postprandial metabolic response and SDA of the *P. vitticeps* of this study are within the range
231 observed for other lizard studies. For lizards feeding on insects, metabolic rates increase by 30 – 340%, and
232 generate an SDA equivalent to 5 – 21% of meal energy [14]. For the only previously published vegetable
233 diet study for lizards, the herbivorous *Angolosaurus skoogi* experienced a 78% increase in metabolic rate
234 following the consumption of carrot meals equaling 7% of body mass [33]. Among mammalian herbivores
235 (e.g., camel, deer, and horse) feeding on straw or hay, metabolic rates increase by 40–100% [14]. Humans
236 tend to exhibit a relatively modest postprandial response, given that experimental mix-nutrient liquid or
237 food diets (~1% of body mass) generate only a 20-40% increase in metabolic rate with SDA equivalent to 7-
238 13% of meal energy [14]. To test the validity of celery as a negative-calorie food, Clegg and Cooper [2]
239 measured the postprandial metabolic response of fifteen female subjects that had each consumed 100 g of
240 celery (16 kcal). Subjects experienced a 33% increase in metabolic rate and generated an SDA (13.8 kcal)
241 that equaled 86% of ingested meal energy.

242 Although there are noted differences in relative meal size and the SDA response between the lizards
243 of this study and any human study, the magnitude of the metabolic increase and the profile of postprandial
244 metabolism are similar [14]. The lizards consumed meals substantially larger, relative to body mass, than
245 humans, and are digesting at a lower body temperature. Therefore the lizards experienced a higher
246 postprandial peak and much longer duration of the metabolic response attributed to meal digestion and
247 assimilation. The seemingly high cost (relative to meal energy) of digesting celery for lizards (this study)
248 and humans [2] stems from the modest amount of energy in the celery meals. When calculated against a low
249 meal energy, any metabolic effort will generate a higher relative cost.

250 It should be noted that the cost of chewing is neither calculated nor incorporated as a component of
251 DIT or SMR [14]. Lizards and humans expend energy masticating raw celery and thus this is an additional
252 cost that reduces the net gain of assimilated energy. For adult human subjects, the chewing of gum resulted
253 in an increase in metabolism of 46 kJ per hour [34]. It took eight women (coauthor AA and students in the
254 lab of SS, mean age of 21.3 years) an average of 5.4 minutes to chew (~400 chews) and swallow 100 g (four

255 intact pieces) of raw celery. Therefore the subjects of the Clegg and Cooper [2] study potentially expended
256 an additional 4 kJ chewing the 100 g of celery that was consumed. Although not accounted for by Clegg and
257 Cooper [2], this added cost erases the net gain assumed in that study.

258 The Clegg and Cooper [2] study as well as the numerous discussions on the legitimacy of negative-
259 calorie foods have focused only on the cost of digestion and assimilation without considering the efficiency
260 at which these food items are digested. Humans consume foods that are easily digested and absorbed due to
261 their highly processed nature and that they generally lack difficult to digest or non-digestible components
262 (e.g., bone, hair, exoskeleton). What is not absorbed in the small intestine (e.g., fiber) is acted upon with in
263 the large intestine by resident microbes (chiefly fermentation of residual carbohydrates) leaving any
264 remaining remnants of the meal to be voided in feces. A traditional approach to quantify the efficiency of
265 digestion has been to subtract fecal energy from meal energy and divide by meal energy [35]. The resulting
266 “digestive efficiency” provides a metric by which comparisons can be made on the relative absorbed gain
267 (or loss) of a meal’s energy. Taking this one step further and also subtracting the energy lost in urine (urate)
268 before dividing by meal energy provides an efficiency index of gained assimilated energy from any meal
269 (“assimilation efficiency”). However, there is an inherent error to this approach because it assumes that all
270 fecal energy is derived from the undigested remnants of the meals.

271 Following the removal of water (~75% of fecal mass), the remaining solids of feces are in fact
272 dominated by bacteria and other microbes (e.g., fungi, virus, protists) and sloughed intestinal epithelial cells,
273 comprising collectively as much as 75% of fecal dry mass [17,36]. To acknowledge the inherent inaccuracy
274 of these calculated efficiencies, they are generally reported as “apparent digestive efficiency (ADE)” and
275 “apparent assimilation efficiency (AAE)” [37,38]. The remaining 25-40% of feces includes lipids (e.g.,
276 bacterial produced short-chain fatty acids) and undigested meal fiber. High fiber diets generate more fecal
277 matter that is of a larger percentage of undigested fiber (relative to bacteria and sloughed cells) [17]. Celery
278 is roughly 40% fiber (dry mass) and for the lizards, as well as for humans, a portion of that fiber is
279 undoubtedly excreted in the feces [18].

280 For a back-of-the-envelope calculation, if we assume (on the high side) that lizard feces are 40%
281 undigested fiber (roughly 30% of the ingested fiber) and add to that the total energy lost in urate (1.06 kJ)
282 and SDA (2.60 kJ), then lizards achieve an assimilated gain of 39.9% of meal energy. This calculated gain
283 is 70% higher than that calculated previously in the Results (Table 3), and theoretically is more accurate.
284 How might this translate to humans digesting celery? For the only human study to assess the cost of celery

285 digestion [2], the projected excreted energy of undigested fiber would most likely exceed the small gain that
286 followed the subtraction of SDA, and the cost of chewing. For this study it is realistic to conclude that the
287 participants did not gain any net calories from their celery meal. However, this study did report an
288 extremely high SDA relative to meal energy compared to other human studies (Secor, 2009).

289
290

291 **Are there negative calorie foods?**

292 For obvious reasons (low calorie, high fiber), celery has been the focus of the only empirical studies
293 to examine the validity of negative-calorie foods [2,15, this study]. While the jury is out on whether for
294 humans the eating of raw celery would result in no net gain of calories; are there other foods that do
295 generate no or negative caloric gain? In absence of determining this for other foods by empirically
296 quantifying the energy lost via feces, urate, and SDA, we can address this question by employing several
297 assumptions of energy loss for each food. First, we set SDA equivalent to 25% of meal energy. While this
298 coefficient is substantially lower than that calculated by Clegg and Cooper [2], it is two to three times
299 greater than that calculated for the majority of human studies, plus it can also account for the cost of
300 chewing [14,34]. Second, the loss of energy in urine is set at 5% of meal energy, which is similar to the loss
301 noted elsewhere [35,37,38]. And third, that the energy lost in feces is 30% of fiber energy. For an
302 additional nine food items commonly listed as negative calorie, the consumption of each (based on these
303 assumptions) results in a net energy gain of roughly 64% of the ingested energy (Table 4). Even if all of
304 fiber energy is lost in the feces (highly unlikely), energy continues to be gained from these foods (~49% on
305 average). Double the loss to SDA and urine along with all fiber energy excreted, they continue to gain
306 energy (~19% on average). As an exercise in budgeting energy, these calculations echo the opinions and
307 discussions of nutritionists, trainers, physicians, and bloggers whom have debunked the existence of
308 negative calorie foods [5,13,16,19].

309

310 **Positive energy gained, negative energy budget**

311 There are however two sides to this story. First, after accounting for the estimated energy expended
312 on chewing, digesting, and assimilating and lost via excretion, all proposed negative-calorie foods can
313 provide a net gain of energy. However, it is important to acknowledge that this gain only stems from the
314 pluses and minuses specific to a meal's digestion and assimilation and does not account for any other

315 metabolic expenses. The other side to this story is how much this gain contributes to supporting other
316 metabolic expenses.

317 The bearded dragons of this study gain theoretically as much as 3.26 kJ from their celery meal over a
318 three-day period while at the same time expending a minimum of 9 kJ (assuming a standard metabolic rate
319 of 0.124 kJ hr⁻¹) fueling their basal metabolism and other non-digestive activities (i.e., moving in their
320 enclosures). Whereas lizards did achieve a positive energy budget specific to digestion and assimilation,
321 they experienced a negative energy budget over those three days (a loss of at least 6 kJ) (Fig. 2). Lizard
322 enthusiasts would never consider celery as a stable diet for bearded dragons, and rather instead feed them
323 higher quality vegetables and greens along with insects [26].

324 The same is undoubtedly true for humans. Those foods touted as negative calorie do generate a net energy
325 gain; however this gain is quickly abolished by the body's own basal rate of metabolism. Consider that a 60
326 kg woman possesses a resting metabolic rate of approximately 220 kJ/h [39]. If we assume that SDA is
327 equivalent to 25% of meal energy (including the cost of chewing) and the woman loses 5% of meal energy
328 in her urine and 30% of fiber energy in her feces, then a celery meal of 5% of body mass (3 kg) would only
329 provide the fuel to cover a little less than six hours of her resting metabolism (Fig. 2). Cut that time in half if
330 she is active. Following these same assumptions, this woman would need to consume daily 9100 kJ or 12.6
331 kg (~28 lbs) of raw celery (given the loss to SDA, feces, and urine) to fuel her resting metabolism for that
332 day. It is unlikely that anyone would maintain a daily diet of 12.6 kg of raw celery, or 9 kg of tomatoes, or
333 even 4.3 kg of raw carrots just to fuel their minimal metabolic needs.

334 The central aim of the majority of weight loss programs is to achieve a negative energy balance; in
335 concept, one's daily energy expenditure (DEE) exceeds their daily metabolizable energy intake (MEI; meal
336 energy minus energy lost in feces and urine). Hence, DEE must be supplemented by the catabolism of
337 endogenous energy stores, chiefly the body's stores of fat. This can be accomplished by increasing
338 expenditure and/or decreasing intake such that $DEE > MEI$. Increasing the relative proportion of the diet
339 that includes low calorie, high fiber foods serve the right side of the equation, reducing MEI. This is the
340 game plan that dominates those diet plans that campaign for the inclusion of proposed negative calorie foods
341 as a surefire means to burn fat and lose weight [5-8,10,11].

342 In this study we empirically tested the theory that a low-calorie, high fiber food would generate an
343 energy deficit due to a cost of processing that exceeds energy gain, and thus be negative caloric. Bearded
344 dragons gained energy from their celery meals (refuting the negative calorie claim), however the energy

345 gained contributes very modestly to fueling their DEE. Rather than labeling such foods as “negative calorie”
346 it would be more accurate to pitch these foods as “negative budget”, the consumption of which will favor a
347 daily negative energy budget, and hence weight loss via the catabolism of body fat.

348

349 **Acknowledgments**

350 This study was conceived following the introduction of the concept of negative calorie foods to the
351 senior author by his son (a sophomore and English major at the time) who proposed to conduct a human
352 study with his friends. However, when it was explained to him the additional need to collect and bomb
353 everyone’s feces, he declined. For assistance with this study, we thank Mimi Bach, Kellen Cowen, Tori
354 Fields, Georgia Gamble, Ayla Jones, Mackenzie Kyler, Alexis McGraw, Zoe Nichols, Anna Reding, and
355 Amanda Shoemaker.

356

357 **Competing interests**

358 No competing interests declared

359

360 **Funding**

361 This work was supported in part by the National Science Foundation (IOS-0466139 to SMS).

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- 456
- 457

458 Table 1. Body mass, standard metabolic rate (SMR), peak $\dot{V}O_2$, scope of peak $\dot{V}O_2$,
459 specific dynamic action(SDA), and SDA coefficient of nine adult beaded dragons (*Pogona*
460 *vitticeps*) that had consumed celery meals equaling in mass to 5% of lizard body mass.

Lizard ID	Mass (g)	SMR (mL O ₂ h ⁻¹)	peak $\dot{V}O_2$ (mL h ⁻¹)	Scope (peak/SMR)	SDA (kJ)	SDA coefficient (%)
PV08	212.8	6.70	10.63	1.59	2.54	33.2
PV20	228.1	5.47	9.92	1.81	2.99	36.5
PV55	210.3	6.73	10.62	1.58	3.00	39.6
PV55,7	201.8	4.44	7.93	1.79	3.27	39.3
PV64	236.2	6.61	10.47	1.58	3.29	38.8
PV70	220.8	7.51	12.74	1.70	2.62	33.0
PV72	190.1	7.03	11.97	1.70	1.35	19.7
PV73	224.7	5.84	11.85	2.03	2.87	35.5
PV74	229.9	7.59	10.30	1.36	1.85	22.4
Mean	217.2	6.44	10.72	1.68	2.64	33.1
SE	4.9	0.34	0.47	0.06	0.22	2.4

461

462

463 Table 2. Dry mass, specific energy, and total energy of feces and urate produced by bearded dragons
 464 (*Pogona vitticeps*) within one week after consuming celery meals equaling 5% of body mass.

Lizard ID	Body mass (g)	Meal mass (g)	Feces mass (g)	Feces specific energy (kJ g ⁻¹)	Feces energy (kJ)	Urate mass (g)	Urate specific energy (kJ g ⁻¹)	Urate energy (kJ)
PV08	206.8	10.34	0.07	17.00	1.19	0.10	11.00	1.10
PV20	229.7	11.48	0.15	16.41	2.46	0.08	11.52	0.92
PV55	209.4	10.47	0.07	14.52	1.02	0.13	9.12	1.19
PV55,7	202.4	10.12	0.08	19.64	1.57	0.07	10.87	0.76
PV64	231.2	11.56	0.13	18.30	2.38	0.09	12.65	1.14
PV70	224.0	11.20	0.19	14.30	2.66	0.13	9.79	1.27
PV72	196.2	9.08	0.15	17.23	2.59	0.14	11.00	1.54
PV73	223.5	11.20	0.14	16.45	2.37	0.12	11.10	1.37
PV74	244.1	12.21	0.25	17.39	4.35	0.02	12.53	0.26
Mean	218.6	10.85	0.14	16.81	2.29	0.11	11.06	1.06
SE	5.2	0.31	0.02	0.56	0.33	0.02	0.38	0.13

465

466

467 Table 3. Total food energy, energy lost to feces, urate and SDA, and remaining net
468 energy (absolute and as a percentage of meal energy) for nine bearded dragons (*Pogona*
469 *vitticeps*) that had consumed celery meals equaling in mass to 5% of lizard mass. Body
470 and meal masses are the same as for Table 2.

Lizard ID	Food energy (kJ)	Feces energy (kJ)	Urate energy (kJ)	SDA (kJ)*	Net energy gained (kJ)	Net gain as % of food energy
PV08	7.47	1.19	1.10	2.48	2.70	36.2
PV20	8.29	2.46	0.92	3.02	1.89	22.7
PV55	7.56	1.02	1.19	2.99	2.37	24.9
PV55,7	7.31	1.57	0.76	2.87	2.10	28.7
PV64	8.35	2.38	1.14	3.24	1.59	19.0
PV70	8.09	2.66	1.27	2.67	1.48	18.3
PV72	6.56	2.59	1.54	1.29	1.14	17.4
PV73	8.09	2.37	1.37	2.87	1.48	18.3
PV74	8.82	4.35	0.26	1.97	2.23	25.3
Mean	7.83	2.29	1.06	2.53	1.89	23.4
SE	0.23	0.33	0.13	0.25	0.17	2.1

471 *SDA was calculated as the product of meal energy and SDA coefficient of Table 1.

472

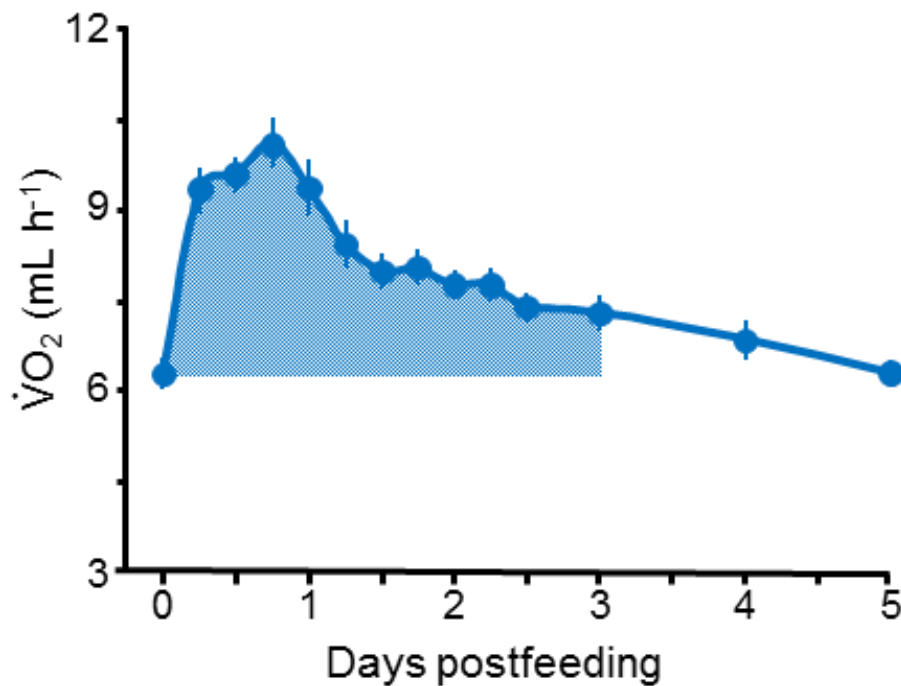
473

474 Table 4. Tabulated for ten commonly listed negative-calorie foods is their percent water content, total
 475 energy per 100 g, total energy partitioned for carbohydrates, fiber, protein and fat, predicted SDA and
 476 energy loss in urine and feces, net gain of energy, and net gain as a percent of total ingested energy.
 477

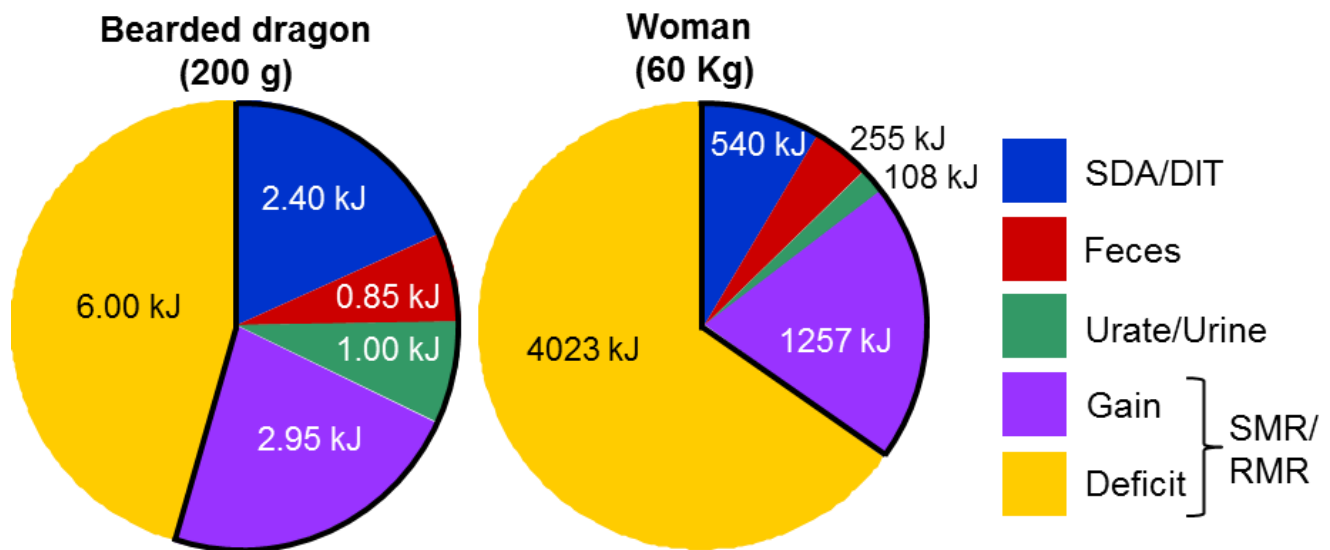
Food	Celery	Broccoli	Apple	Carrot	Grapefruit	Tomato	Cucumber	Watermelon	Green leaf lettuce	Blueberries
% water	95.4	89.3	85.6	88.3	88.1	94.5	96.7	91.5	95.0	84.2
kJ*/100 g	71.5	182.4	254.7	195.0	207.2	92.3	55.0	149.9	81.0	281.7
Carbs (kJ)	52.2	116.7	242.7	168.4	187.3	68.4	38.0	132.7	50.4	254.6
Fiber (kJ)	28.2	45.8	42.3	49.3	28.2	21.1	12.3	7.1	22.9	42.3
Protein (kJ)	12.4	50.7	4.7	16.7	13.8	15.8	10.6	11.0	24.5	13.3
Fat (kJ)	6.8	14.7	6.7	9.5	5.6	7.9	6.4	6.0	6.0	13.1
SDA (kJ)	17.9	45.6	63.7	48.8	51.8	23.1	13.8	37.5	20.3	70.4
Loss in urine (kJ)	3.6	9.1	12.7	9.8	10.4	4.6	2.8	7.5	4.1	14.1
Loss in feces (kJ)**	8.4	13.7	12.7	14.8	8.5	6.3	3.7	2.1	6.9	12.7
Net gain (kJ)	41.6	113.9	165.6	121.7	136.6	58.3	34.8	102.8	49.8	184.5
Net gain % of food kJ	58.2	62.5	65.0	62.4	65.9	63.1	63.3	68.6	61.5	65.5

478 *Energy is presented in kilojoules. To convert to kilocalories, divide by 4.18. **Energy lost in feces
 479 assumes that all ingested sugars, protein, and fat are absorbed and that 30% of fiber energy is lost in feces.
 480

481 Figure 1. **Postprandial profile of oxygen consumption and accumulative SDA.** Postprandial
482 profile of oxygen consumption (mean and SE) and accumulative SDA for nine adult bearded
483 dragons for five days after consuming a meal of diced raw celery equivalent in mass to 5% of
484 lizard mass. Oxygen consumption rates peaked at 24 hours postfeeding at a mean value that was
485 62% greater than standard metabolic rate (time = 0). The shaded area represents the extra oxygen
486 consumed above standard metabolic rate for the three-day duration of elevated metabolism from
487 which SDA was quantified.
488



489 **Figure 2. The partitioning of meal energy and energy deficit from celery meals.** Pie charts illustrating
490 the energy partitioned to SDA/DIT, feces, urate/urine, and metabolizable gain (outlined in black) for raw
491 celery meals equaling in mass to 5% of body mass over a 3-day period for 200-gram bearded dragon and
492 over a 1-day period for 60-kg woman. The total energy of each chart represents the predicted expenditure on
493 standard metabolic rate (SMR, at 30C) for the bearded dragon for 3 days and on resting metabolic rate
494 (RMR) for a woman for 1 day. The noted deficit for each is the amount of additional energy needed to fuel
495 SMR or RMR for 3 days and 1 day, respectively, for lizards and women, beyond that gained from the celery
496 meal (deficit + gain = SMR or RMR). The fuel to cover this deficit undoubtedly originates from
497 endogenous body stores.



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499