

1 **Micronutrient status and intake in omnivores, vegetarians and vegans in Switzerland**

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16

17 **Abstract**

18 *Purpose:* Vegetarian and vegan diets have gained popularity in Switzerland. The nutritional
19 status of individuals who have adopted such diets, however, has not been investigated. The
20 aim of this study was to assess the intake and status of selected vitamins and minerals
21 among vegetarian and vegan adults living in Switzerland.

22 *Methods:* Healthy adults (omnivores (OV), nOV=100; vegetarians (VG), nVG=53; vegans
23 (VN), nVN=53) aged 18 to 50 years were recruited and their weight and height were
24 measured. Plasma concentrations of the vitamins A, C, E, B1, B2, B6, B12, folic acid,
25 pantothenic acid, niacin, biotin and β -carotene and of the minerals Fe, Mg and Zn and
26 urinary iodine concentration were determined. Dietary intake was assessed using a 3-day
27 weighed food record and questionnaires were issued in order to assess the physical activity
28 and lifestyle of the subjects.

29 *Results:* Omnivores had the lowest intake of Mg, vitamin C, vitamin E, niacin and folic acid.
30 Vegans reported low intakes of Ca and a marginal consumption of the vitamins D and B12.
31 The highest prevalence for vitamin and mineral deficiencies in each group were as follows:
32 in the omnivorous group, for folic acid (58%); in the vegetarian group, for vitamin B6 and
33 niacin (58% and 34%, respectively); and in the vegan group, for Zn (47%). Despite negligible
34 dietary vitamin B12 intake in the vegan group, deficiency of this particular vitamin was low in
35 all groups thanks to widespread use of supplements. Prevalence of Fe deficiency was
36 comparable across all diet groups.

37 *Conclusions:* Despite substantial differences in intake and deficiency between groups, our
38 results indicate that by consuming a well-balanced diet including supplements or fortified
39 products, all three types of diet can potentially fulfill requirements for vitamin and mineral
40 consumption.

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42 Key words: vegetarian, vegan, vitamins, minerals, dietary intake, micronutrient status

43

44 **Introduction**

45 Because they abstain from consuming meat and fish, vegetarians predominantly consume
46 plant products. The so called ovo-lacto-vegetarians avoid all meat and fish, but do consume
47 eggs and dairy products. Vegans follow a stricter form of vegetarianism and thus avoid all
48 animal products [1].

49 In Switzerland, a growing proportion of the population follows a vegetarian diet. According to
50 the 2012 Swiss health survey [2], 2.7% of the investigated subjects (women 3.9%, men
51 1.3%) never consume meat or sausages, showing a steady increase from 1.9% in 1992. At
52 the same time, the percentage of people consuming meat daily decreased from 32.7% in
53 1992 to 23.9% in 2012 (based on personal communication with the Federal Office of
54 Statistics). In the Nutri-Trend-Study in 2000, 9% of those surveyed stated that they “almost
55 always” followed a vegetarian diet [3]. The Swiss Association of Vegetarians estimates that
56 about 3% of the population are vegetarian - a conclusion based on a number of surveys
57 between 1997 and 2013. Although no exact data are available, it is estimated that about
58 10% of Swiss vegetarians adhere to a vegan diet and the majority of the remaining 90%
59 follows an ovo-lacto-vegetarian eating pattern [4].

60 Various studies have previously reported differences in nutrient intake in vegetarians and
61 vegans as compared to omnivores [5-8]. In general, total energy intake does not seem to
62 differ significantly between the 3 groups but the contribution of proteins to the total energy
63 intake is typically lower in the non-meat-eating diet groups. Total fat intake, expressed as a
64 percentage of total energy, is lowest in vegans and highest in omnivores. Furthermore,
65 meat-eaters consume by far the highest amounts of saturated fatty acids. On the other hand,
66 the intake of polyunsaturated fatty acids is substantially higher in vegans than in the other
67 two groups, particularly in meat-eaters. Vegans have also been found to have the highest
68 fiber intakes while omnivores have the lowest values. In the EPIC-Oxford study [9], the
69 vegan group was reported to have the highest consumption of vitamin B1, folate, vitamin C
70 and vitamin E and the lowest intakes of retinol, vitamin B12 and vitamin D. Regarding
71 mineral consumption, vegans showed the highest intakes of Mg and Fe and the lowest
72 intakes of Ca and Zn. However, Appleby et al. did not find significant group differences for
73 the intake of vitamin B2, niacin, vitamin B6 and potassium [9].

74 Several studies have evaluated the health benefits associated with vegetarian eating
75 patterns as compared to omnivorous diets. Convincing and consistent evidence exists
76 regarding lower rates of coronary heart disease (CHD), colorectal cancer, obesity, and
77 diabetes. Total cancer incidence appears to be slightly lower, and life expectancy greater in
78 vegetarians as compared to the general population [10]. The observed positive health effects

79 may not only be due to the avoidance of meat and fish; a diet rich in vegetables, fruits, nuts
80 and whole grain products is known to contribute to better health, independent of the
81 consumption of meat and fish [11]. Moreover, it is widely acknowledged that vegetarians
82 often have healthier lifestyles due to adequate physical activity and lower alcohol and
83 tobacco consumptions [1].

84 On the other hand, changing from an omnivorous to a vegetarian or even to a vegan diet
85 may lead to deficiencies of some vitamins, minerals and trace elements, as nutrients
86 contained in high amounts in animal products may be difficult to replace. Of particular
87 importance is vitamin B12, which occurs in substantial amounts only in foods derived from
88 animals. The same is true for vitamin D, which can however be synthesized in human skin
89 under light exposure. Other nutrients of concern are Zn, Fe, Se, Ca and the long-chain n-3-
90 fatty acids [1].

91 Therefore, the aim of this study was to investigate micronutrient status and intake in healthy
92 subjects following an omnivorous, a vegetarian or a vegan diet and to investigate
93 associations between health status and intake.

94

95 **Subjects and Methods**

96 *Subjects*

97 For this study, 206 healthy adult female and male subjects between the age of 18 and 50
98 years who have been following either a vegan (VN), vegetarian (VG) or omnivorous (OV)
99 diet for at least 1 year prior to the study, were recruited. We recruited 53 ovo-lacto-
100 vegetarians, 53 vegans and 100 omnivores. OV and VG subjects taking antibiotics or
101 vitamin/mineral supplements, as well as pregnant and lactating women were excluded from
102 the study. Vitamin/mineral supplementation is very common among VNs. As it was
103 impossible to recruit the required number of vegan subjects otherwise, the criteria regarding
104 supplementation were relaxed for this group. VN subjects who were regularly consuming
105 supplements were included in this group, but supplementation had to be interrupted for at
106 least 14 days prior to the start of the study. The usual supplementation was documented.
107 Any subject who was suffering from a chronic disease or had surgery less than 3 months
108 prior to the study was excluded. Signed written informed consent was obtained from each
109 subject. The study has been approved by the ethical committees of ETH Zurich and of the
110 Canton of Vaud.

111 Subjects were recruited in the area of Lausanne by the Swiss Vitamin Institute (Epalinges,
112 Switzerland) as well as in the area of Zurich by ETH Zurich (Zurich, Switzerland) using
113 advertisements in schools, restaurants and shops.

114 *Study Design*

115 The subjects had only one meeting with the investigators which took place on the first day of
116 the study. After an overnight fast, the subjects came to the ISV in Epalinges or to ETH in
117 Zurich where their body weight and height were measured and a spot urine sample (for the
118 determination of iodine status) was collected. Subsequently, a venous blood sample of
119 approximately 25 ml was drawn into three heparinized tubes for the determination of
120 micronutrient status including the concentration of the vitamins A, C, E, B1, B2, B6, B12,
121 folic acid, pantothenic acid (B5), niacin (B3), biotin (B7) and β -carotene as well as several
122 minerals and trace elements, namely Fe, Zn, Se and Mg. In the following week, each subject
123 collected three additional spot urine samples. The samples were sent to ETH Zurich and
124 stored at -20°C until determination of the iodine status was performed.

125 *Assessment of vitamin and mineral status*

126 Plasma concentrations of the vitamins A, C, E, B1, B2, B6, B12, folic acid, pantothenic acid
127 (B5), niacin (B3), biotin (B7) and β -carotene were measured at the ISV in Epalinges. For the
128 determination of vitamin A and E concentrations, HPLC with a UV detector was used (Bui,
129 1993). Vitamin C was measured by HPLC with an electrochemical detector [12]. Isocratic
130 liquid chromatography was applied to assess β -carotene concentration [13]. The vitamins
131 B1, B2, B6, B12, pantothenic acid, niacin, biotin and folic acid were measured using
132 microbiologic methods on microplates with different bacterial strains [14].

133 Urinary iodine concentration was measured in duplicate at the ETH using a modification of
134 the Sandell-Kolthoff reaction [15]. Each of the 4 samples collected per person was measured
135 individually and the mean was used for subsequent data analysis. Four samples were
136 collected per person to account for within-subject variations between days. Fe status was
137 assessed using the parameters hemoglobin (Hb), plasma Ferritin (PF) and C-reactive
138 protein (CRP). Immediately after blood collection, Hb concentration was analyzed using an
139 electronic counter (HemoCue, Angelholm, Sweden). The measurements were carried out at
140 either the ISV or ETH. PF and CRP were analyzed at ETH using an automated
141 chemiluminescent immunoassay system (Immulite 2000, Siemens Healthcare Diagnostics
142 Inc., Tarrytown, New York, USA). If CRP values were >5 mg/l, the samples were excluded
143 from further Fe status analysis. Determination of Mg and Zn concentration in plasma was
144 carried out at ETH Zurich by flame atomic absorption spectrometry (FAAS). Se analysis was

145 performed at the Federal Office of Public Health, Bern (Bundesamt für Gesundheit, BAG)
146 using coupled plasma mass spectrometry (ICP-MS).

147

148 *Assessment of dietary intake*

149 To assess dietary intake, a three-day weighed food record was done. This dietary protocol
150 was completed over three non-consecutive days during the week after the day of blood
151 collection by the participants at home. The sampling period included two week days and one
152 weekend day. Participants were provided with kitchen scales for weighing their consumed
153 food in order to reduce measurement errors. The completed food records were returned to
154 ETH where dietary intake was calculated using the software EBISpro for Windows. Food
155 records were excluded if under- or overreporting was apparent. The Goldberg cut-offs for
156 over- and underreporting were applied [16].

157 *Assessment of lifestyle factors*

158 In addition to the evaluation of food intake and micronutrient status, subjects were asked to
159 fill in 2 questionnaires at home assessing lifestyle factors such as their physical activity and
160 their consumption of alcohol and tobacco. Physical activity was evaluated using the
161 International Physical Activity Questionnaire (IPAQ). Alcohol and tobacco consumption were
162 evaluated by means of a questionnaire similar to the one used in the Swiss Health Survey
163 [17].

164 *Data analysis*

165 Statistical analysis was carried out using the statistical package IBM SPSS Statistics 20 for
166 Windows. Non-normally distributed variables were log-transformed prior to analysis. The
167 weighed food records were analyzed using the nutrition software EBISpro for Windows (4.0,
168 Dr. Jürgen Erhardt, Universität Hohenheim, Germany), which allows calculation of energy as
169 well as the macro- and micronutrient intakes of each individual. All protocols were tested for
170 over- and underreporting ($REE \times 2.4$ and $REE \times 1.2$, respectively) prior to further analysis. One-
171 way ANOVA with post-hoc Bonferroni correction was used to assess differences in
172 biochemical as well as dietary intake parameters and physical activity scores between the
173 three groups. The Chi-square test was used to calculate whether the prevalence of
174 micronutrient deficiency, micronutrient status above the reference range, low micronutrient
175 intake and smoking were the same across the three diet groups. To assess continuous
176 associations between dietary intake and biochemical parameters, Pearson correlation was
177 used. Spearman's rho correlation was used to calculate correlations between nonparametric

178 data (e.g. tobacco and alcohol consumption). The level of significance for all analysis was
179 set at $p < 0.05$.

180

181 **Results**

182 *Subject characteristics*

183 A total of 206 subjects participated in the study. **Table 1** shows the basic characteristics of
184 the study population by diet group. The groups did not differ in terms of gender distribution
185 ($p = 0.712$) or age ($p = 1.000$). BMI was significantly lower in the VN group only when
186 compared to OVs ($p = 0.016$).

187 The questionnaires regarding lifestyle and physical activity were returned by 196 and 195
188 subjects, respectively. Ninety-nine percent of all subjects judged their general health status
189 as good or very good. The majority of subjects (64% of OV, 74% of VG and 61% of VN)
190 declared themselves to be health conscious, and this score correlated negatively with
191 alcohol consumption ($r = 0.245$, $p = 0.001$) and positively with total physical activity ($r = 0.149$,
192 $p = 0.039$). Of the total study population, 24.2% were current smokers with no difference
193 between the groups. Alcohol consumption was more frequent in the OV and VG groups with
194 36.6% and 34.7% reporting to consume alcohol 1-2 times per week, respectively. In
195 comparison, only 15.4% of VNs reported the same consumption. Similarly, alcohol
196 abstinence was most frequent in the VN group (28.8% compared to 3.2% for OV and 6.1%
197 for VG). Total physical activity was not significantly different between the 3 groups, nor were
198 any of the sub-categories of physical activity (work, transport, domestic, and leisure time
199 activity).

200 *Micronutrient status and prevalence of micronutrient deficiencies*

201 There were some missing values in the vitamin and mineral analysis due to insufficient
202 sample volume or technical problems during analysis. The following measurements could
203 not be carried out: β -carotene status for one OV subject, vitamin C for one VG subject, PF,
204 Mg and Zn status for seven OV subjects and UIC status for seven OV, three VG and one VN
205 subject. A total of 12 female OV, four male OV and one male VN subjects had to be
206 excluded from further analysis of PF data due to elevated CRP values ($CRP > 5\text{mg/l}$).

207 Vitamin and mineral status is reported in **Table 2** which also indicates significant differences
208 between groups. While OV subjects showed the highest status in PF, Zn, iodine, vitamin A,
209 vitamin E and niacin, VN participants had the highest status in Mg, Vitamins C, B1, B6 and
210 folic acid. **Table 3** shows the percentage deficiency for each assessed parameter by diet

211 group including the normal ranges used. As a substantial amount of the VN subjects was
212 consuming vitamin B12 supplements, their vitamin B12 status was calculated separately.
213 Median vitamin B12 status of vegans consuming B12 supplements was 342 pmol/l (27-5166)
214 compared to 274 pmol/l (24-2632) in those reporting no consumption of supplements. In the
215 vegan group consuming no supplements three subjects had vitamin B12 concentrations
216 below the cut-off for sufficiency (150 pmol/l) whereas only one subject was deficient in the
217 group consuming supplements.

218 *Dietary intake*

219 Out of all 206 three-day weighed food records, 194 (94%) were returned to ETH Zurich and
220 analyzed ($n_{OV}=93$, $n_{VG}=49$, $n_{VN}=52$). Underreporting occurred in a total of 31 cases ($n_{OV}=16$,
221 $n_{VG}=6$, $n_{VN}=9$) which were excluded from further intake analysis. Over-reporting was not
222 found in any case. Table 4 describes macronutrient and energy intakes reported, and Table
223 5 shows the intake of micronutrients ($n_{OV}=77$, $n_{VG}=43$, $n_{VN}=43$). Energy and fat intake did not
224 differ significantly between the groups, but protein intake was higher in the OV group
225 compared to the two others ($p<0.001$). Carbohydrate intake was significantly higher in the
226 VN group ($p<0.01$). The intake of fiber was highest in the VN and lowest in the OV group
227 ($p<0.0001$). Ca intake was highest in the OV group, while the intakes of Mg, Fe and K were
228 highest in the VN group. Similarly, vitamin D and vitamin B12 intakes were highest in the OV
229 group, while the intakes of vitamin E, C, B1, B6, niacin and folic acid were highest in the VN
230 group.

231 *Correlation between dietary intake and biochemical status analysis*

232 To investigate the associations between dietary intake of a nutrient and the respective
233 micronutrient status, Pearson correlations were used. No significant associations between
234 dietary intake and status was determined in any of the three diet groups for retinol, vitamin
235 C, vitamin E, vitamin B1, vitamin B2 and vitamin B12. Mg intake was significantly positively
236 correlated with Mg status in the OVs ($r=0.288$, $p=0.011$) but not the 2 other groups (VG:
237 $r=0.056$, $p=0.723$; VN: $r=0.118$, $p=0.451$). Iron intake was positively correlated with PF in the
238 OV ($r=0.247$, $p=0.030$) and the VG ($r=0.331$, $p=0.030$) groups but not the VN group
239 ($r=0.168$, $p=0.281$). Zn intake was also significantly positively correlated with Zn status in OV
240 subjects ($r=0.301$, $p=0.008$) but not in the two other groups (VG: $r=0.040$, $p=0.800$; VN:
241 $r=0.020$, $p=0.901$). Significant positive correlations between intake and status in all groups
242 were identified for vitamin B6 (OV: $r=0.460$, $p<0.001$; VG: $r=0.304$, $p=0.048$; VN: $r=0.427$,
243 $p=0.004$) and folic acid (OV: $r=0.298$, $p=0.009$; VG: $r=0.447$, $p=0.003$; VN: $r=0.302$,
244 $p=0.049$).

245 **Discussion**

246 As VG and especially VN lifestyles are currently emerging in western societies, it is
247 important to understand the impact of those dietary patterns on micronutrient status and
248 thereby health. Health benefits of VG and VN diets have been discussed in association with
249 cardiovascular disease and certain cancers, but their impact on the supply of certain
250 micronutrients is of concern. To our knowledge, this is the first study investigating the status
251 and intake of a broad range of micronutrients, including both vitamins and minerals, in OVs,
252 VGs and VNs in Switzerland.

253 *Nutrients with highest status in omnivores*

254 We found PF to be significantly higher in our OV study population, despite the highest iron
255 intakes in the VNs. The phenomenon of high iron intake in VNs has been reported previously
256 by Davey et al. (2003) and Janelle and Barr (1995) [5, 18]. A lower status despite high
257 intakes can mainly be explained by the lower iron bioavailability from plant foods compared
258 to animal foods. Generally, non-heme iron is much less well-absorbed compared to heme
259 iron found in meat. In addition, most plant foods contain certain iron-absorption inhibitors
260 such as polyphenols or phytic acid which reduce the bioavailability of iron even further [19].
261 The fact that we found PF to be positively correlated with iron intakes in the OV and the VG
262 groups but not the VN group can be explained by the fact that the consumption of iron
263 absorption inhibitors was highest in the VN group.

264 In contrast to previous studies indicating higher zinc intake in OVs compared to VGs or VNs
265 [5, 18, 20], we found similar zinc intake in all 3 groups. Nevertheless, zinc status was
266 significantly lower in VGs and VNs as compared to OVs, which is in line with a previous
267 study by Krajčovičová-Kudláčková et al. [21]. Similarly to iron, there was a significant
268 positive association between zinc intake and zinc status in the OV group, but not in the VG
269 or VN groups. Zinc bioavailability also depends on the presence of absorption inhibitors and
270 enhancers [22]. Consequently, zinc-rich plant foods such as legumes, whole grains, nuts
271 and seeds - which are consumed in higher amounts by VGs and VNs - also contain high
272 amounts of phytic acid, a zinc absorption inhibitor [23].

273 As dietary iodine in Switzerland is largely supplied by iodized salt, assessment of iodine
274 intake using dietary assessment is near to impossible. However, urinary iodine provides a
275 reliable measure of individual iodine intake if collected repeatedly. However, ideally a higher
276 number of samples per subject than used in the current study would have been desirable
277 [24]. We found a significantly lower urinary iodine concentration in VNs as compared to the
278 two other groups. This result confirms findings of Krajčovičová-Kudláčková et al. (2003) and

279 Remer et al. (1999), both of whom reported a significantly lower urinary iodine concentration
280 in VGs and particularly in VNs as compared to OVs [25, 26]. With a median of 56 µg/l, the
281 urinary iodine concentration of our VN population was far below the current WHO cut-off for
282 iodine sufficiency of 100 µg/l. This may be due to the fact that VNs do not consume milk and
283 dairy products, which are, besides iodized salt, one of the most important dietary iodine
284 sources in Switzerland [27].

285 Because the most common dietary sources for calcium are milk and dairy products, VNs
286 were expected to have low calcium intakes. This has also been demonstrated in several
287 previous studies [5, 18, 20, 28]. In agreement with those findings, we found that the VN
288 subjects in our study consumed significantly lower amounts of calcium compared to the
289 other groups. Fifty four % of the VN subjects consumed less Ca than recommended by the
290 EAR (800 mg/d), as compared to 28% of OVs and 17% of VGs. Serum calcium is tightly
291 regulated and only in extreme circumstances concentrations below or above the normal
292 range can be detected [29]; consequently, it is not a good indicator of calcium nutritional
293 status. For this reason, we had to rely solely on intake assessment for this mineral. Low-
294 calcium intake is considered a risk factor for the development of osteoporosis [30] and it is
295 thus important that VNs are informed regarding alternative calcium sources and/or
296 supplements.

297 Serum retinol is determined by both retinol and β-carotene intake. While preformed retinol is
298 found predominantly in animal foods such as liver, butter, cream, cheese, fish and eggs, all
299 β-carotene is of plant origin. High amounts are found in carrots, herbs, green leafy
300 vegetables, apricots, mangos, pepper etc. [31]. The total intake of retinol equivalents (RE) is
301 usually calculated as 1 RE = 1 µg retinol = 12 µg β-carotene [32]. We could not demonstrate
302 differences in the intake of RE between the three diet groups which is supported by some
303 [28], but not all, previous studies [18, 33]. Nevertheless, serum retinol status was
304 significantly higher in the OV group as compared to the VN group. As the conversion factor
305 of β-carotene to RE is very low and has further been suggested to depend on the cell's
306 vitamin A needs [34], it is highly likely that the lower serum retinol status found in VNs is due
307 to the fact that they consume no foods of animal origin and therefore also consume no
308 preformed vitamin A. Our findings seem to indicate that a general conversion factor of 12:1
309 for β-carotene to RE is too high. Nevertheless, plasma retinol concentrations in our subjects
310 were mainly in the normal range with <10% showing deficiencies, even in the VN subjects.
311 Thus, β-carotene intake seem to be sufficient in this setting.

312 Despite higher intakes of vitamin E (composed of α-, β-, γ-, and δ-tocopherol) in VGs and
313 VNs, vitamin E status was found to be highest in our OV group. Vitamin E acts as an

314 antioxidant for polyunsaturated fatty acids [35]. As those fatty acids were consumed at
315 higher amounts by our VN subjects, this may explain the higher use and thus lower status of
316 vitamin E in this group. Furthermore, in order to judge vitamin E status correctly, one should
317 also determine cholesterol levels. This was not done in our current study and we can
318 therefore not determine the α -tocopherol:cholesterol ratio. As α -tocopherol is mainly
319 transported in low density lipoproteins, which are thus protected from oxidation, the α -
320 tocopherol:cholesterol ratio is an important indicator for oxidative damage [36]. Cholesterol
321 intakes and plasma cholesterol are generally higher in OVs as compared to VGs and VNs
322 and thus, despite higher absolute plasma vitamin E concentrations in our OV subjects, we
323 expect their α -tocopherol:cholesterol ratio to be lower as compared to the two other groups.
324 This has been demonstrated in a previous study by Krajčovičová-Kudláčková et al. (1995,
325 1996) [35].

326 Similar to Davey et al. (2003) we found the lowest niacin intakes in our VG participants and a
327 significantly-higher niacin consumption in OVs and VNs [5]. In other studies [18, 28, 33],
328 significantly-lower niacin intakes in VNs as compared to OVs were reported. Despite the
329 high intakes in VNs, their niacin status was still significantly lower compared to the OVs and
330 was similar to the VGs. Consequently, 30% of all VGs and VNs were found to be niacin
331 deficient. However, in the human body niacin can be converted not only into the
332 metabolically active form of nicotinamide adenine dinucleotide (NAD) but also the amino acid
333 tryptophan, and the classical deficiency symptoms of pellagra only occur in a deficiency of
334 both niacin and tryptophan. Thus, there is no direct need for concern in our study population
335 [34].

336 As plant foods generally do not contain any vitamin B12, VGs - and particularly VNs - were
337 expected to show lower intakes and plasma concentrations of this nutrient. In accordance
338 with many other studies [5, 18, 20, 28, 33, 37, 38], we found very low dietary vitamin B12
339 intakes in VNs, higher but still low intakes in VGs and an appropriate vitamin B12 supply
340 from diet only in OVs. Because no supplements were consumed by the subjects throughout
341 the duration of the study, habitual use of supplements was not assessed in the dietary
342 record. However, we have asked all subjects at the beginning of the study whether they
343 consume supplements on a regular basis and if yes, what kind of supplements. Of our vegan
344 subjects, 43% reported that they normally consume supplements containing vitamin B12 but
345 we did not assess exact frequencies of consumption. This was reflected by the median
346 plasma vitamin B12 concentrations determined in our subjects, which was similar and in all
347 groups. Similarly, the prevalence of vitamin B12 deficiency was low in all groups,
348 nevertheless with four out of 53 subjects it was highest in the vegan group. However, even
349 when analyzing only the group which abstained from taking regular vitamin B12

350 supplements, their status was still relatively high (median of 274 pmol/l vs. 342 pmol/l in the
351 group taking supplements) and only three subjects were B12 deficient. This can be
352 explained by the fact that the human body only requires relatively low amounts of vitamin
353 B12; body stores are therefore depleted slowly [39]. Throughout the 1-3 years during which
354 our subjects followed their diets, they may not have completely depleted their vitamin B12
355 stores despite not having taken supplements. Furthermore, we did only as for supplements
356 taken on a regular basis and some subjects may only take supplements irregularly. In
357 addition, not all fortified products may have been captured by the dietary record.
358 Furthermore, several plant foods such as algae, edible mushrooms and fermented products
359 have been reported to contain significant amounts of vitamin B12, even though their
360 biological activity and consequential effect on B12 status is inconclusive [40]. It may well be,
361 that some processed foods specifically made for vegans contain such ingredients which
362 again were not captured by our dietary software. In contrast to our work, several previous
363 studies reported lower vitamin B12 status in both VG and VN subjects [20, 28, 37-39],
364 including a recent comprehensive review [41]. Despite the low prevalence of clinical vitamin
365 B12 deficiency in our study it has to be considered, that the so called sub-clinical vitamin
366 B12 deficiency is not well defined and its impact unclear [42, 43]. On the other hand, it was
367 reported, that a serum vitamin B12 concentration of <148 pmol/l has a sensitivity to diagnose
368 97% of true, clinical deficiency [43].

369 We have not been able to assess vitamin D status in this study but have only examined
370 intake. However, as vitamin D - or rather its active metabolite 1,25-dihydroxyvitamin D3 - can
371 be synthesized in the skin if exposed to sunlight [44], vitamin D intake is not expected to
372 clearly determine status. As dietary sources of vitamin D are generally animal foods, the
373 finding of lower vitamin D intake in our VG and VN groups is not unexpected and is also in
374 line with previous data [5, 28]. Overall, vitamin D intake was low in all three diet groups
375 which may indicate a potential risk for low vitamin D status especially in winter time. During
376 this season vitamin D synthesis in the skin is not possible in Switzerland due to the
377 irradiation angle of the sun [45]. The low vitamin D intake together with the low calcium
378 intake observed in the VN group may potentially put them at risk for the development of
379 osteoporosis. This clearly is a factor which should be closely observed especially in the VN
380 group.

381 *Nutrients with highest status in vegans*

382 Similar to other studies [5, 18], we have found highest magnesium intakes in the VN group.
383 Furthermore, in our study population, the highest magnesium status was seen in the same
384 group, a finding not confirmed by all previous studies [21, 46]. Nevertheless, we have only

385 found a significant positive correlation between magnesium intake and status for the OV
386 subjects. Two main factors can help explain this finding: 1) magnesium is absorbed at higher
387 proportions from low-magnesium diets [47, 48] (in our case the OV diets); 2) dietary factors
388 such as phytic acid and oxalate can inhibit magnesium absorption [49]. These may explain
389 the differences in correlations found between groups as well as the relatively small
390 differences in status compared to intake.

391 In line with many other studies [5, 18, 20, 28, 33], we found the lowest vitamin C
392 consumption in OVs, intermediate consumption in VGs and the highest intake in VNs with
393 significant differences between all groups. Accordingly, the lowest mean vitamin C status as
394 well as the highest prevalence of vitamin C deficiency (12%) occurred in the OV group.
395 Similarly, in earlier studies, Krajčovičová-Kudláčková et al. (1995, 1996) also measured
396 plasma vitamin C concentration in VG and non-VG subjects and reported significantly higher
397 values for VGs [21]. Despite this, in our data, calculated vitamin C intakes did not correlate
398 with status. However, because storage, processing and preparation can destroy more than
399 50% of the vitamin C content of a fresh product [34], and because vitamin C content
400 depends also on variety and ripeness (especially in fruits), it is very difficult to precisely
401 assess vitamin C intake with dietary assessment methods This may explain the lack of
402 correlations found in this study.

403 In the present study, vitamin B1 intake and status were significantly higher in VNs compared
404 to the other diet groups. Similar results were reported in previous studies [5, 18, 33, 38], but
405 not all agree [28]. Sprouts, seeds and nuts, which are the best sources for vitamin B1, most
406 likely account for the high vitamin B1 consumption in VNs, whereas OVs can benefit from
407 high vitamin B1 concentrations in pork [31]. Despite intake and status showing similar results
408 in our subjects for vitamin B1, no correlations could be shown between the two. This may be
409 due to the fact that the dietary records were completed in the week after blood sampling and
410 that vitamin B1 has a relatively short half-life [34]. Thus, the intake reflected in the measured
411 plasma concentration may not have been the same as the one assessed in the food records.

412 Vitamin B6 is contained in many different foods. Good sources are wheat germs, sprouts,
413 soy beans, whole oats, pulses, liver, chicken, pork, fish and beef [31]. Sufficient vitamin B6
414 intake could thus be obtained in all examined diets. Even though the mean vitamin B6
415 consumption was adequate in all groups, VNs showed significantly-higher vitamin B6 intakes
416 in comparison to OVs and VGs. This finding is in agreement with two studies by Haddad et
417 al. and Majchrzak et al. who also report highest vitamin B6 intakes in the VN group [33, 38];
418 however, other studies do not agree [5, 18, 28, 37]. We found vitamin B6 status to be
419 significantly lower in VGs compared to the two other groups, with a vitamin B6 deficiency of

420 almost 60% in this group. This finding is in contrast to some [38, 39], but not all, previous
421 studies [37]. It can likely be explained by the fact that VGs do not consume meat - one of the
422 major sources of vitamin B6 in OVs - but also consume germs, sprouts and soy beans -
423 other good vitamin B6 sources - in lower extents than VNs.

424 Yeast, germs, pulses, bran, sprouts, liver, green vegetables and nuts are good sources of
425 folic acid [31]. It is thus not surprising that we found highest intakes of folic acid in VNs,
426 intermediate intakes in VGs and the lowest intakes in OVs; this is in agreement with the
427 results of several other studies [5, 18, 28, 33, 38]. Accordingly, folic acid status was also
428 found to be significantly higher in the VN group compared to the two others - a finding in line
429 with most earlier studies [20, 37, 38], but not all [50]. Based on the low intake and status of
430 folic acid, 58% of the OVs in our study were diagnosed with folic acid deficiency compared
431 with only 13% of our VN population.

432 *Nutrients with no differences amongst groups*

433 We found no significant differences between groups in selenium, vitamin B2, panthotenic
434 acid and biotin status. As all those micronutrients can be found in a variety of food groups
435 not specific to any of the three diet groups, this finding was not unexpected.

436 *Lifestyle*

437 The common assumption that VGs and particularly VNs follow a healthier lifestyle could not
438 be confirmed in the present study. With a mean of 24% of all subjects, the smoking rate in
439 our study was relatively high compared to that in previous studies (3-18%) [5, 6, 51-53].
440 However, this high smoking rate is not uncommon for Switzerland: in a study from 2010 27%
441 of 14-65 year-olds and 39% of 20-24 year-olds reported to smoke cigarettes [54]. Alcohol
442 abstinence on the other hand was found to be more prevalent in our VN group (28.8%
443 compared to 6.1% in VG and 3.2% in OV). This shows a similar tendency as previous
444 reports showing that up to 73% of VNs never consume alcohol [51-53]. However, this is the
445 only factor where VNs seemed to be more health-conscious compared to the subjects in the
446 other diet groups. Physical activity in any domain was not found to be significantly different
447 among groups. However, it can of course be argued that volunteers of the OV group who
448 participate in nutrition and lifestyle studies are usually more health-conscious than the
449 average population [5, 7]. This so-called "healthy volunteer effect" may explain why no
450 significant differences between OV, VG and VN participants could be detected in the present
451 study. Besides, we need to consider that our study sample, especially the OV group, was
452 clearly not a representative sample of the total population and therefore the general
453 applicability of these results is limited. A further limitation in the VN group may be that we

454 cannot rule out the possibility that there was a certain difference between those who
455 consume dietary supplements and those who don't. Even though we did not find significant
456 differences between the two groups in their general characteristics, some subtle differences
457 (e.g. regarding health consciousness) which we could not detect may exist.

458 In conclusion, it can be said that all diet groups show low intakes and deficiencies for some
459 micronutrients. Therefore, based on our results (and focusing only on micronutrients), it is
460 impossible to define which of the three types of diet would be most beneficial to human
461 health. Irrespective of the type of diet a person chooses, it is important for them to be well
462 aware of its potential limitations and to take the corresponding precautions (for example by
463 taking vitamin B12 supplements). Fortified products may also be a good alternative to
464 supplements in all diet groups as they may help cover the needs for the different nutrients.

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475

476

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609

610 **Table 1:** Characteristics of all subjects participating in a cross-sectional study in Switzerland by diet group

	OV (n _{OV} =100)	VG (n _{VG} =53)	VN (n _{VN} =53)
Gender distribution [% females]	63 ^a	68 ^a	60 ^a
Age ¹ [y]	31.6 (±9.4) ^a	30.8 (±7.6) ^a	29.9 (±7.3) ^a
BMI ¹ [kg/m ²]	23.0 (±3.1) ^a	22.5 (±2.8) ^{a,b}	21.6 (±2.5) ^b
Duration of vegetarian/vegan diet ² [y]		9.5 (1.0-30.4)	3.0 (1.0-18.0)

611 OV=omnivore, VG=vegetarian, VN=vegan

612 ¹ mean ± SD (all such values)

613 ² median (min-max) (all such values)

614 Values not sharing a common superscript letters are significantly different from each other (p<0.05)-

615

616

617 **Table 2:** Mineral and vitamin status of all subjects participating in a cross-sectional study in Switzerland by diet
618 group.
619

Nutrient	OV	VG	VN
Normal range (m/f)			
Mg [mmol/l] 0.7-1.1 mmol/l	0.86±0.08 ^{1,a}	0.85±0.07 ^a	0.93±0.07 ^b
Hemoglobin [g/l] 140-180/120-160 g/dl	145±14 ^a	146±12 ^a	147±11 ^a
Plasma ferritin [ng/ml] 15-300 µg/l	58 (3-463) ^{2,a}	32 (7-184) ^b	40 (9-277) ^{a,b}
Zn [µg/dl] 74-130/70-130 µg/dl	85±12 ^a	78±9 ^b	72±10 ^c
Iodine [µg/l urine] 100-300 µg/l	83 (22-228) ^a	75 (1-610) ^a	56 (27-586) ^b
Selenium [µg/l] 70-150 µg/l	94.0±12.5 ^a	90.3±14.2 ^a	90.1±21.9 ^a
Vitamin A [nmol/l] 920-2760 nmol/l	1869±436 ^a	1599±389 ^b	1562±408 ^b
β-Carotene [nmol/l] 600-4700 nmol/l	3617±1286 ^a	3839±1087 ^a	4137±1456 ^a
Vitamin E [µmol/l] 13.0-36.2 µmol/l	26.0±5.8 ^a	22.7±4.5 ^b	22.1±4.3 ^b
Vitamin C [µmol/l] 11.1-49.7/35.4-80.1 µmol/l	55.4±17.2 ^a	68.9±23.3 ^b	71.8±23.4 ^b
Vitamin B1 [nmol/l] 7-50 nmol/l	30.7±12.1 ^a	29.4±8.9 ^a	36.4±11.5 ^b
Vitamin B2 [nmol/l] 50-120 nmol/l	92.0±44.8 ^a	82.4±42.4 ^a	79.8±41.7 ^a

Vitamin B6 [nmol/l]	22 (7-113) ^a	16 (2-70) ^b	27 (9-365) ^a
18-200 nmol/l			
Vitamin B12 [pmol/l]	340 (147-3207) ^a	361 (28-1744) ^a	289 (24-5166) ^a
150-790 pmol/l			
Niacin [nmol/l]	580 (0-1156) ^a	398 (35-1487) ^b	416 (185-839) ^b
130-410 nmol/l			
Folic acid [nmol/l]	14.3 (1.9-42.3) ^a	16.6 (7.4-39.8) ^a	25.7 (4.2-88.3) ^b
15-57 nmol/l			
Panhotenic acid [nmol/l]	336 (142-924) ^a	361 (125-766) ^a	319 (121-744) ^a
200-500 nmol/l			
Biotin [nmol/l]	1.4±0.95 ^a	1.5±0.77 ^a	1.6±0.71 ^a
0.7-1.8 nmol/l			

620

621 OV=omnivore, VG=vegetarian, VN=vegan

622 ¹ mean±SD, all such values

623 ² median (min-max), all such values

624 Means and medians not sharing a common superscript letter are significantly different from each other (ANOVA with post-hoc bonferroni test, p<0.05)

626 The sample consisted of 100 OV, 53 VG and 53 VN. The following measurements could not be carried out: β-carotene status for 1 OV subject, vitamin C for 1 VG, PF, Mg and Zn for 7 OV subjects and UIC for 7 OV, 3 VG and 1 VN subject. A total of 12 female OV, 4 male OV and 1 male VN had to be excluded from further analysis of PF data due to elevated CRP values (CRP >5mg/l).

630 .

631 **Table 3:** Percentage of subjects below cut-off for deficiency in the individual micronutrients and anemia,
 632 respectively in a cross-sectional study in Switzerland by diet group.
 633

	Normal range (m/f)	OV	VG	VN
Mg	0.7-1.1 mmol/l	2.2 ^a	3.8 ^a	0.0 ^a
Hb ¹	140-180/120-160 g/dl	2.0	0.0	0.0
Plasma ferritin	15-300 µg/l	14.3 ^a	11.3 ^a	13.5 ^a
Zn	74-130/70-130 µg/dl	10.8 ^a	18.9 ^a	47.2 ^b
Iodine	100-300 µg/l	64.5 ^a	66.0 ^a	78.8 ^a
Vitamin A	920-2760 nmol/l	1.0 ^a	0.0 ^a	3.8 ^a
β-Carotene ¹	600-4700 nmol/l	1.0	0.0	0.0
Vitamin E	13.0-36.2 µmol/l	0.0 ^a	0.0 ^a	3.8 ^a
Vitamin C	11.1-49.7/35.4-80.1 µmol/l	12.0 ^a	3.8 ^a	3.8 ^a
Vitamin B1 ¹	7-50 nmol/l	0.0	0.0	0.0
Vitamin B2	50-120 nmol/l	14.0 ^a	22.6 ^a	26.4 ^a
Vitamin B6	18-200 nmol/l	29.0 ^{a,c}	58.5 ^b	24.5 ^c
Vitamin B12	150-790 pmol/l	1.0 ^a	5.7 ^a	7.5 ^a
Niacin	130-410 nmol/l	11.0 ^a	34.0 ^a	26.4 ^a
Folic acid	15-57 nmol/l	58.0 ^a	30.2 ^b	13.2 ^b
Pantothenic acid	200-500 nmol/l	6.0 ^a	13.2 ^b	7.5 ^b
Biotin	0.7-1.8 nmol/l	16.0 ^a	15.1 ^a	7.5 ^a

634
 635 OV=omnivore, VG=vegetarian, VN=vegan
 636 ¹ statistics cannot be computed because parameter is constant
 637 Values not sharing a common superscript letter are significantly different from each other (chi square test with
 638 bonferroni correction for multiple comparisons; p<0.05)

639 The sample consisted of 100 OV, 53 VG and 53 VN. The following measurements could not be carried out: β-
 640 carotene status for 1 OV subject, vitamin C for 1 VG, PF, Mg and Zn for 7 OV subjects and UIC for 7 OV, 3 VG
 641 and 1 VN subject. A total of 12 female OV, 4 male OV and 1 male VN had to be excluded from further analysis of
 642 PF data due to elevated CRP values (CRP >5mg/l).

643

644

645 **Table 4:** Average daily energy, macronutrient, cholesterol, sugar and dietary fiber intake of all subjects
 646 participating in a cross-sectional study in Switzerland by diet group
 647

	OV	VG	VN
N	77	43	43
Energy [kcal]	2319±500 ^{1,a}	2263±559 ^a	2469±596 ^a
Protein [g]	85±24 ^a	64±21 ^b	65±21 ^b
Protein [%]	15±3 ^a	12±2 ^b	11±2 ^b
COH [g]	259±84 ^a	267±70 ^a	324±102 ^b
COH [%]	45±8 ^a	48±8 ^a	54±12 ^b
Fat [g]	94±26 ^a	93.1±27 ^a	96±46 ^a
Fat [%]	36±6.8 ^a	36±7 ^a	33±12 ^a
SFA [g]	37±12 ^a	34±11 ^a	20±12 ^b
MUFA [g]	35±11 ^a	35±11 ^a	41±24 ^a
PUFA [g]	12±5 ^a	15±7 ^a	25±13 ^b
Cholesterol [mg]	301 (97-699) ^{2,a}	200 (23-713) ^b	12 (0-81) ^c
Sugar [g]	126±58 ^a	131±48 ^a	180±115 ^b
Fiber [g]	20±10 ^a	31±12 ^b	52±18 ^c

648

649

650 OV=omnivore, VG=vegetarian, VN=vegan

651 COH=carbohydrates, SFA=saturated fatty acids, MUFA=monounsaturated fatty acids, PUFA=polyunsaturated
 652 fatty acids

653 ¹ mean ± SD, all such values

654 ² median (min-max), all such values

655 Means and medians not sharing a common superscript letter are significantly different from each other (one way

656 ANOVA with post-hoc bonferroni correction; p<0.05)

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658 **Table 5:** Average daily micronutrient intake in all subjects participating in a cross-sectional study in Switzerland by diet group and estimated average requirements (EAR) for
 659 dietary intake of those nutrients [55].

	EAR (m/f)	OV	VG	VN
Ca [mg] ¹	800	1022±330 ^a	1116±434 ^a	817±285 ^b
Mg [mg] ¹	330/255	350±96 ^a	448±162 ^b	702±255 ^c
P [mg] ¹	580	1331±356 ^a	1359±517 ^a	1427±462 ^a
Fe [mg] ²	6/8.1	11.8 (7.2-43.3) ^a	14.7 (7.7-44.3) ^b	22.9 (12.8-43.0) ^c
Zn [mg] ¹	9.4/6.8	11.2±3.7 ^a	10.2±4.5 ^a	11.5±4.1 ^a
K [mg] ¹	2000	3063±946 ^a	3440±1022 ^a	5375±2178 ^b
Na [mg] ¹	550	2848±978 ^a	2936±1284 ^a	2994±1481 ^a
Cl [mg] ¹	830	4625±1553 ^a	4633±1575 ^a	4801±2057 ^a
Retinol equ. [µg] ^{2,3}	625/500	775 (182-2986) ^a	967 (453 -1998) ^a	739.41 (167-6280) ^a
Vitamin D [µg] ²	10	1.5 (0.2-10.0) ^a	1.2 (0.1-4.0) ^b	0.1 (0-0.9) ^c
Vitamin E equ. [mg] ^{1,4}	12	13.2±4.8 ^a	17.6±8.8 ^b	25.5±9.2 ^c
Vitamin C [mg] ²	75/60	94 (24-356) ^a	158 (39-706) ^b	239 (70-1873) ^c
Vitamin B1 [mg] ²	1/0.9	1.4 (0.5-4.2) ^a	1.3 (0.5-19.4) ^a	2.1 (0.9-5.7) ^b
Vitamin B2 [mg] ²	1.1/0.9	1.7 (0.9-4.5) ^a	1.5 (0.9-4.4) ^a	2.0 (0.8-3.7) ^a
Vitamin B6 [mg] ²	1.3/1.1	2.0 (0.7-8.3) ^a	1.9 (0.8-6.0) ^a	2.9 (1.7-8.0) ^b
Vitamin B12 [µg] ²	2	4.1 (0.9-14.7) ^a	1.6 (0.3-14.4) ^b	0.2 (0-6.4) ^c
Niacin [mg] ²	12/11	18.9 (6.1-64.1) ^a	12.5 (4-39.1) ^b	17.6 (6-36.2) ^a
Folic acid [µg] ²	320	281 (127.2-897.0) ^a	368 (170-2194) ^b	662 (294-2107) ^c

Panhotenic acid [mg] ²	6	5.5 (2.1-11.4) ^a	5.5 (2.9-14.5) ^{a,b}	6.4 (2.9-21.3) ^b
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661 ¹ mean±SD, all such values

662 ² median (min-max), all such values

663 ³ retinol equivalent, (1 retinol equivalent = 1µg retinol = 12µg β-carotene)

664 ⁴ α-tocopherol equivalent (1 mg α-tocopherol =1.25 mg α-tocopherol equivalents)

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666 Means and medians not sharing a common superscript letter are significantly different from each other (one-way ANOVA with post-hoc bonferroni correction; p<0.05)

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