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The association of mycoprotein-based foods consumption with diet quality, energy intake and non-communicable diseases' risk in the UK adult population using the National Diet and Nutrition Survey (NDNS) years 2008/09-2016/17: A cross-sectional study

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Short title: Association of mycoprotein with health and nutrient intake

Abstract:

Mycoprotein is a fungal-based ingredient rich in fibre and protein used in meat-replacement foods sold under the name of Quorn in 17 countries. Fibre and protein positively regulate glycaemia, lipidaemia, energy intake which are non-communicable diseases' (NCDs) markers. We performed a cross-sectional study to investigate the association of mycoprotein intake with diet quality, nutrient, energy intake and NCDs risk within UK free-living adults from the National Diet and Nutrition Survey (NDNS) from years 2008/09-2016/17. Dietary approaches to stop hypertension (DASH) and healthy diet index (HDI) were calculated to estimate diet quality. Comparison between mycoprotein consumers (>1% kcal) and non-consumers, and associations between consumers and nutrient intakes, NCDs' risk markers and diet quality were investigated using a survey-adjusted general linear model adjusted for sex, age, body mass index (BMI), ethnicity, socio-economic, smoking status, region of residency, total energy, energy density, HDI and non-mycoprotein fibre intake. 5507 adults were included, of which 3.44% were mycoprotein consumers and had a higher intake of dietary fibre (+22.18%,p<0.001), DASH score (+23.33%) and HDI (+8.89%) (p<0.001, both) and lower BMI (-4.77%,p=0.00) vs. non-consumers. There was an association (p=0.00) between mycoprotein consumers and diet quality scores (+0.19 and +0.26), high fibre (+3.17g), total and food energy (+3.09 and +0.22 kcal), but low energy density intakes (-0.08 kcal/g,p=0.04). Consumers were negatively associated with fasting blood glucose (-0.31 mmol/L,p=0.00), and glycated haemoglobin A1c (HbA1c) (-0.15%,p=0.01). In conclusion, mycoprotein intake is associated with lower glycaemic markers and energy density intake, and high fibre, energy intake and diet quality scores.

Keywords: Mycoprotein, cross-sectional analysis, energy intake, diet quality, non-communicable disease markers

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Introduction

Non-communicable diseases (NCDs) such as cardiovascular disease and type 2 diabetes are the leading cause of global mortality in developed countries. Metabolic changes such as raised blood pressure, blood glucose and cholesterol as well as being overweight/obese are associated with increased risk of NCDs, all of which can be modified by lifestyle[1]. An important lifestyle modification to decrease the risk of developing NCDs is diet. Dietary protein has been shown to modulate blood glucose response by stimulating insulin release via yet unclear mechanisms[2], and to decrease energy intake associated to increased satiety signals[3]. However, current industrialised nations mainly rely on animal-based protein and such production practices are detrimental to the environment through fossil fuel usage, animal methane production, effluent waste, water, and land consumption[4]. High red and processed meat intake is positively associated with diabetes, cardiovascular disease, weight gain, total mortality, and its substitution of plant-protein was associated with lower mortality[5,6]. Therefore, sustainable meat alternatives with similar or healthier nutrition value are to be explored. On the other hand, dietary fibre intake (mainly from plant sources which have a lower environmental impact compared to meat and dairy[7] has been shown to decrease blood glucose via delayed gastric emptying and gut microbiota fermentation[8]. Total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) have also been shown to decrease following interventions with fibre possibly via mechanisms such as decreased insulin secretion of hepatic cholesterol synthesis, prevention of bile salt reabsorption and increased short-chain fatty acid production[9]. Furthermore, dietary fibre intakes reduce the energy density of foods, and delays gastric emptying, subsequently decreasing appetite, energy intake and ultimately modulating body weight[10]. In this context, mycoprotein is a fungal-based ingredient characterised by being high in both dietary fibre (66% fungal beta-glucan and 33% chitin) and protein, and low in energy (Table 1). The protein content of mycoprotein has been shown to be as bioavailable as whole milk, chicken and eggs, and to stimulate muscle anabolism to similarly to leucine-matched milk protein (ref Edwards, Monteyne, Dunlop). Mycoprotein-based foods are produced much sustainably in comparison to animal-derived proteins. For example, compared to beef mince, Quorn mince uses 89% less carbon (16.21 vs. 1.74 kg CO_2e kg^{-1}), 94% less land (0.0068 vs. 0.00038 ha kg^{-1}) and 90% less water (L kg^{-1})[11].

Mycoprotein-based foods are commercially available under the brand name of Quorn in 17 countries across the world, and is growing in popularity in the UK as meat replacements[12]. A recent systematic review suggested that an acute intake of mycoprotein increases insulin sensitivity and decreases energy intake in healthy lean and overweight individuals compared to matched animal-derived protein[13]. In addition, chronic mycoprotein interventions have resulted in a reduction in low-density lipoprotein cholesterol (LDL-C) and total cholesterol (TC) in both controlled and free-living settings[14-16]. Although the mechanisms underpinning these effects are poorly understood, it is possible that the high fibre and protein content of mycoprotein may have contributed to these effects, although literature on the effects of fungal-derived chitin and beta-glucan on blood glucose and lipids in humans is very scarce. Interestingly, it has been recently suggested that the unique food structure of mycoprotein (known as hyphae) may act as a mechanism underpinning the metabolic effects by acting in the upper gastrointestinal tract. *In vitro* evidence suggests that the hyphal structure can trap digestive enzymes such as amylase and bile salts during digestion, consequently decreasing starch hydrolysis and lipolysis, and hindering the digestion, absorption and appearance in blood of carbohydrates and lipids[17]. Other mechanisms underpinning the effects of mycoprotein on appetite and potentially on blood glucose control, is the possibility that insoluble parts of mycoprotein (such as the fibre portion) can act as a substrate for gut microbiota fermentation and stimulate the production of short-chain fatty acids, linked to glucose metabolism and appetite central regulation[18], as shown in one *in vitro* study using healthy stool samples[19].

These studies investigated the effect of mycoprotein-based foods (referred hereon as mycoprotein) on NCDs' risk factors such as blood glucose, lipid profile and energy intake. However, the majority of these studies were well-controlled studies, primarily of an acute and controlled nature, which do not represent a free-living setting. We therefore investigated the association between mycoprotein intake and diet quality, energy and nutrient intake as well as NCDs' risk factors using data from a nationally representative cohort to provide insight into the impact of mycoprotein consumption on NCDs' risk in a free-living setting.

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Methods

A cross-sectional analysis was performed and reported in line with the STROBE-Nut checklist [31].

<u>The National Diet and Nutrition Survey Rolling Programme (NDNS-RP) and study population</u>

The National Diet and Nutrition Survey Rolling Programme (NDNS-RP) is a long-term governmental survey scheme to assess nutrient intake of free-living population households in the UK (England, Scotland, Wales and Northern Ireland) that are 1.5 years old or above. Between 2008 and 2017, people from the UK were randomly selected and invited to complete a 4-consecutive day food diary, an interview (dietary habits, socio-demographic background and lifestyle), have anthropometric measurements undertaken, and to provide overnight fasting blood (for non-diabetics, otherwise non-fasting samples were obtained) and urine samples. Food diary data was processed by trained coders and entered into the Medical Research Council human nutrition research dietary assessment system called "Diet In Nutrients Out"[20]. Food composition from the Department of Health's NDNS Nutrient Databank was used to calculate macro- and micronutrient intake. Composite items such as sandwiches and home-made meals were coded as individual foods. Two quality controls on the food diary coded data were performed by editors independent of the coders. The first was aimed to identify missing data fields and assess the feasibility of maximum and minimum portion sizes entered within each subsidiary food group. This was undertaken on a random subsample (10% of total diary sample). The second quality control was aimed to identify extreme high or low nutrient intakes (outside the 2.5 and 97.5 percentiles for their age and sex group as based on previous NDNS data). This quality check was performed by the editors, with extreme values being checked against the food diary entries. Further details on the NDNS-RP methodology are described elsewhere[21]. In this study, the mean of the 4-consecutive days of reported dietary data was calculated and used for the analysis. The NDNS-RP was conducted according to the Declaration of Helsinki guidelines and ethical approval was obtained by the local Research Ethics Committees of the areas covered by the survey. All participants gave informed consent. The cross-sectional analysis hereby reported includes data from adult participants (≥18 years old, N=5507), who participated in the study in the years 1-9 of the NDNS-RP (2008-2017).

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NCDs' risk markers

NCDs' risk markers such as body mass index (BMI) (kg/m^2), systolic and diastolic pressure (mmHg), TC (mmol/l), triacylglycerides (mmol/l), HDL-C (mmol/l), low density lipoprotein LDL-C (mmol/l), TC to HDL ratio, C-reactive protein (CRP) (mg/l), fasting blood glucose (mmol/l) and glycated haemoglobin A1c (HbA1c,%) were included in this analysis. Body weight and height was measured using a portable stadiometer and a weight scale which allowed the calculation of the BMI by the field researchers. Trained researchers took blood pressure from volunteers who rested in a sitting position after a 5-minute rest. Measurements were taken in three occasions using Omron HEM907, and the mean value of second and third value readings was used for analysis. Protocol for blood sample collection, processing, analysis and quality controls are described elsewhere[22]. Missing data for blood pressure, BMI and blood analytes were omitted and only available data was included in the analysis. The sample size for each blood pressure and blood analyte are reported in the Tables reporting associations in the Results section.

Diet quality assessment

The quality of the diet was assessed by generating an indicator and a score based on the already existing healthy diet indicator (HDI)[23] and the dietary approaches to stop hypertension (DASH) score[24]. HDI was developed in 1997 reflecting the WHO dietary recommendations (1990) aimed at preventing the development of chronic diseases. HDI has been associated negatively with overall and NCDs' mortality[25], but not with cancer risk[26]. HDI includes nutrients and foods such as saturated fatty acids, poly unsaturated fatty acids, protein, complex carbohydrates, fibre, fruit, vegetables, pulses, nuts, seeds and total sugar score. DASH was established in 1995 with the initial aim to reduce blood pressure. DASH score[27] contemplates 8 target nutrients such as total fat, saturated fat, protein, fibre, cholesterol, calcium, magnesium and potassium. DASH scores ≥ 4.5 are considered to be DASH concordant. Cholesterol intake data was missing in the NDNS-RP database. For this reason, this nutrient was omitted in its calculation, therefore for this study, DASH concordant score is considered to be ≥ 3.5.

Mycoprotein-based foods energy (kcal), weight (g) intake and mycoprotein consumers (>1% kcal of 4-consecutive day average energy intake from mycoprotein) were quantified prior to analysis. A quartile segregation of mycoprotein consumers was omitted because of the small

sample size of mycoprotein consumers (n=191). Mycoprotein-based foods labelled with the name "Quorn" included: dippers, goujons, burgers, mycoprotein and pieces, quarter pounder grilled, sausage, mini eggs, sliced meats for all years (1-9); in addition to vegetarian sausage roll, pie, bacon style strips, cottage pie for most years (1-8), as well as breadcrumbs including nuggets, stir fried, lasagne for years 1-4.

Statistical analysis

Normality of the data was checked visually from the normal residual distributions Q-Q plots. Normally distributed data are presented as mean and standard error of the means (SE) and non-normally distributed data are presented as medians and interquartile range (IQR) for demographic variables, and as survey adjusted means and standard error of the means (SE) for nutrients, diet quality scores and NCDs' risk factors for both non- and consumers of mycoprotein-based foods. Measures on blood samples, anthropometric and blood pressure data were not available for all participants; however, comparisons and associations of these measurements with mycoprotein consumption were analysed only for the remaining participants described in the summary Tables in the Results section. To assess the association between mycoprotein consumption and demographic variables a survey-adjusted general linear model (GLM) was used. To assess the associations between mycoprotein consumption and nutrients, diet quality scores and NCDs' risk factors a survey-adjusted GLM with predictors for age, sex, body mass index (BMI), ethnicity, region of residency, socio-economic and smoking status, and alcohol, energy density, total energy and fibre (except fibre from mycoprotein) intakes, and HDI was performed. For the association with alcohol, fibre, energy intake and energy density the above adjusted model was not including the covariates alcohol, fibre, energy intake and energy density, respectively. Colinearity of variables was checked using variance inflation factor, values less than 10 were considered not colinear. Squared root transformation of skewed variables was performed. To assess differences between consumers and non-consumers independent t tests were performed for the survey adjusted means and SE. Survey weight factors for individuals (wti_), nurses (wtn_) and blood measurements (wtb_) were used for each specific survey year to control for any potential selection bias in the associations with nutrient intake, diet quality and body weight (wti_ used), blood pressure (wtn_ used) and blood markers (wtb_ used). Preprocessing of the data was performed using R Studio 1.3.1056 using the tidyverse, tidyr, reshape2 and dplyr packages and statistical analysis was performed using the survey and

svydiags package. A two-sided p-value of ≤0.05 was considered as statistically significant. A sensitivity analysis was performed to identify the differences between participants with full dataset compared to those with missing data for blood, BMI and blood pressure. A t-test considering unequal variance and on-way ANOVA was performed. The results are described in the Supplemental Materials Tables S1- S6.

Results

Demographic characteristics

Overall, 3.44% of the adult UK sample population have been identified as mycoprotein consumers, of these 28.27% are vegetarian, 0.5% are vegan and 71.73% are omnivores. The 96.56% of the adult UK sample population have been identified as non-mycoprotein consumers, and of these, 1.42% were vegetarian, 0.15% vegan and 98.41% omnivores. The percentage of mycoprotein consumers in the population varied from survey year to year, with an overall 21.50% increase in percentage of consumers from year 2008/09 to 2016/17 (Figure 1). The change in total diet energy obtained from mycoprotein intake, the total grams of mycoprotein, total energy from mycoprotein and energy density from mycoprotein intake in the mycoprotein consumer group over the survey years is described in the Supplementary Table S7. The median age in mycoprotein consumers was lower than in non-mycoprotein consumers (-7 years). In mycoprotein consumers, the proportion of females was considerable higher than of men (+24%) whereas in non-consumers the difference is smaller (+16%). White ethnicity was predominant over other ethnicities for both mycoprotein consumers (95%) and non-consumers (92%) and most of the population was representative of England region (67% and 59%, respectively). A higher proportion of mycoprotein consumers had higher (+6.3%) or lower managerial (+6.2%) professional occupations compared to non-consumers. A fewer proportion of smokers were found to be mycoprotein consumers compared to the non-consumers (-9%). The survey-adjusted generalised linear model showed that mycoprotein consumption in adults was significantly associated with age (p<0.001) and smoking status (p=0.02). The demographic characteristics for both non- and mycoprotein-based foods adult consumers, as well as the associations between mycoprotein consumer group and demographic characteristics are shown in Table 2.

Nutrient intake and diet quality

Overall, the median consumption of mycoprotein in consumers are 127.4 kcal or of 112.48 grams, contributing to a 7.84% of the total energy (Table 2). The survey-adjusted means showed that mycoprotein consumers had a significantly higher intake CN6 poly unsaturated fatty acids (+6.46%, p=0.04), carbohydrate (+4.45%, p<0.001), starch (6.56%, p>0.00), fibre (+22.18%, p<0.001), and lower intake of protein (-8.71%, p=0.00) and alcohol (-31.61%, p=0.04) relative to non-consumers. HDI median and DASH score were statistically significantly greater for mycoprotein consumers by +8.98% and +23.33%, (p<0.001, both). The other variables were not statistically significantly different between the two groups. The nutritional intake for both non- and mycoprotein-based foods adult consumers are shown in Table 3.

The associations between the mycoprotein consumer group and nutrient intake and diet quality are shown in Table 3. Mycoprotein consumers were statistical significantly associated with slightly increased food energy (kcal) (+0.22, p<0.001) and total energy (kcal) (+3.09, p<0.001), but reduced energy density (food kcal/gram) (-0.08, p<0.04). Furthermore, positive statistical significantly associations between mycoprotein consumers and specific nutrients were found for poly unsaturated fatty acids CN6 (CN6PUFA) (+0.57%, p<0.04), CN3PUFA (+0.10%, p<0.04), starch (+1.97%, p<0.04), fibre (g) (+3.17, p<0.001). There were statistical significantly associations between mycoprotein consumers and reduced intake of total sugars (-1.75%, p<0.001), non-milk extrinsic sugars (-3.20%, p<0.001), free sugars (-2.83%, p<0.001) and alcohol intake (g) (-1.35, p<0.001). For healthy diet scores, mycoprotein consumers were statistically significantly associated with increased HDI (+0.19, p<0.001) and DASH score (+0.26, p<0.001), when adjusted for age, sex, BMI, ethnicity, region of residence, socio economic and smoking status, alcohol, energy intake, energy density and non-mycoprotein fibre intake.

NCDs' risk markers

The NCD's risk markers for both non- and mycoprotein-based foods adult consumers are shown in Table 4. Mycoprotein consumers had a lower BMI compared to non-consumers which was statistically significantly different ($-4.77 kg/m^2$, p=0.00). Other markers such as fasting blood glucose and HbA1c were lower in the mycoprotein consumer group but it did not reach statistical significance. The remaining NCD's risk markers studied were not shown to be statistically significantly different between the two groups.

The associations between the mycoprotein intake and NCDs' risk markers are shown in Table 4. Being a mycoprotein consumer was almost significantly associated with a lower BMI (-0.80 kg/m^2, p=0.05) when adjusted for age, sex, ethnicity, region of residency, socio-economic and smoking status, alcohol, non-mycoprotein fibre, energy density, total energy intake and HDI. Mycoprotein consumers were statistical significantly associated with lower fasting blood glucose (FBG) (-0.31 mmol/L, p=0.00) and HbA1c (-0.06%, p=0.01) when adjusted for age, sex, BMI, ethnicity, region of residency, socio-economic and smoking status, alcohol, non-mycoprotein fibre, energy density, total energy intake, and HDI. There was no statistically significant association between mycoprotein consumption and the rest of NCDs' markers when adjusted for the aforementioned cofounders.

Discussion

Acute and chronic intervention studies suggest that mycoprotein may have a positive effect on glycaemia, insulinaemia, lipidemia, appetite regulation and energy intake[13-16]. Nevertheless, the intake of mycoprotein in the free-living UK population and its association with demographics and nutritional profiles as well as with health parameters has never been investigated. In the present study, we aimed to investigate the association between mycoprotein intake and diet quality, energy, nutrient intake as well as NCDs' risk factors. The present cross-sectional study showed that mycoprotein-consumers had a statistical significantly higher intake of dietary fibre, total energy, DASH scores and HDI and lower BMI compared to non-consumers. Being a mycoprotein consumer was significantly positively associated with HDI, DASH score, fibre, PUFA, total and food energy intake, but negatively associated with energy density, sugars, and alcohol intake, as well as lower FBG and HbA1c.

Being a mycoprotein consumer was associated negatively with energy density (kcal/g) intake. This finding is in contrast with the results of an acute feeding study in which overweight pre-menopausal females increased their intake choice of high energy density foods such as high fat/high sugar and low fat/high sugar foods, following mycoprotein intake compared to tofu and similarly to chicken[28]. However, this study was performed in very controlled settings, potentially influencing participant's choices in a free-living setting. Moreover, our results also showed that despite the negative association with energy density intake, mycoprotein-consumer group has a slightly increased total and food energy intake. Altogether, this suggests increased eating rate in this group, which could be linked to a lack of a satiating

macronutrients intake, such as protein, for which mycoprotein consumers's intake was 8.71% less than non-mycoprotein consumers. On the other hand, mycoprotein consumers obtained on average 22% more dietary fibre, which is also known to be a satiating macronutrient, although it is possible that overall, mycoprotein-consumers did not consume enough amount of satiating macronutrients, explaining their increased total energy intake. Our findings are surprising since mycoprotein is rich in dietary protein and fibre - two macronutrients that have a role at suppressing appetite and energy intake[28,31-32] via a myriad of mechanisms. Clinical data from controlled studies has shown a consistent effect between acute mycoprotein intake (mean intake of 102.1g) and reductions in appetite, energy intake post-24h and at *ad libitum*[31-32]. Energy intake is an important determinant of NCDs' risk, especially if it surpasses energy expenditure and it results in increased body weight and BMI. We also showed that on average mycoprotein-consumers had a lower BMI compared to non-consumers, and BMI was almost significantly negatively associated with this consumer group. The more favourable BMI tendency suggests that the mycoprotein group may be more physically active, despite an association with higher energy intake, but this was not possible to assess as a standard measurement for such physical activity was missing for some survey years.

Overall, diet quality scores such as HDI and DASH score were positively associated with being a mycoprotein consumer and were significantly higher in the mycoprotein group by 8 and 23%, respectively. This suggests that mycoprotein consumers in general have a healthier choice of foods, including foods aimed at modulating the risk for hypertension, which is a NCD's risk marker. In particular, the nutrient intake mycoprotein consumers were associated with were with higher starch and CN3- and CN6PUFA. Resistant starch (the fraction of starch that resists digestion and constitutes fibre) has been positively linked to improved NCDs' risk factors such as glycaemia, insulinaemia, bowel health, blood lipid profile, and increased satiety and reduced energy intake[33]. However, NDNS data does not provide details for resistant starch intake, opening an avenue for research. PUFA intake has been consistently shown to have favourable effects in glycaemic control, insulin resistance and insulin secretion[34], and to be linked to improving cardiometabolic health[35]. Moreover, alcohol intake was negatively associated with being a mycoprotein consumer, possibly explaining the significantly lower tendency of an increased BMI in this group.

The mycoprotein consumer group had a significantly higher intake of dietary fibre (+22%) compared to non-consumers, and being a consumer was significantly associated with a 3.71g

increase in fibre intake. Considering that mycoprotein-based foods have on average 6% dietary fibre, it is estimated that mycoprotein intake contributed to the 29% of the total dietary fibre intake, giving a 6.74g median increase in fibre intake in the consumer group. Mycoprotein consumers were estimated to eat 23g of fibre/day on average, being closer to the Scientific Advisory Committee on Nutrition recommendations for fibre of 30g/day[36], compared to the non-consumers who had an average fibre intake of 17g/day. This indicates that mycoprotein could possibly represent an important dietary element to increase one's intake of fibre to meet the current dietary fibre intake daily recommendations. A recent systematic review and meta-analysis of epidemiological studies and RCTs with people with T2D showed that dietary fibre intake improved glycaemic control, blood lipids, body weight, and inflammation, as well as a reduction in premature mortality, which are all linked to NCD's risk prevention[37]. In line with this, in this study we have reported that mycoprotein consumers were associated with lower FBG. There is controversy in regard to the validity of FBG as a clinical marker for NCDs' risks[38], and other glycaemic measures such as oral glucose tolerance test or Hb1Ac are better at predicting NCDs' risk[39]. Even though, oral glucose tolerance test data was not available in the NDNS database, HbA1c available data has shown to be strongly negatively associated with being a mycoprotein consumer, when adjusted for all cofounders, including healthy diet. These associations with glycaemia could be partly attributed to the effect of both the fibre content of mycoprotein and the unique hyphal food structure. Acute human trials have shown that while there is not a significant effect on postprandial blood glucose following mycoprotein intake, a reduced postprandial insulin is evident compared to macronutrient matched-chicken or soy[31,40]. Further, analysis have shown significant increase in insulin sensitivity following doses ranging 44-132 g of mince mycoprotein in overweight and obese humans. However, it seems that these effects were not replicated in one chronic study where meat and fish was replaced for mycoprotein for 7-days in healthy participants[41]. Although, the current evidence from human trials is small and heterogenous, a recent *in vitro* study exploring the behaviour of mycoprotein during digestion as a mechanism underpinning postprandial metabolic response following mycoprotein intake, showed a proportional inhibition of starch hydrolysis with increasing doses of mycoprotein. Moreover, using fluorescent microscopy, it was observed a physical entrapment of amylase enzyme within the hyphal structure, suggesting that during digestion mycoprotein may delay carbohydrate digestion and absorption, reducing glycaemic response[17].

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The data from the present study showed that 3.44% of the surveyed adult participants consumed mycoprotein-based foods (an average of 147 g/day, equivalent to 2 servings of mycoprotein and representing an average of 7.84% of their total energy intake). However, the 4-day food diaries used in this study did not allow to differentiation between regular mycoprotein consumers to those "occasional" mycoprotein consumers. A 21.50% increase in total mycoprotein consumers was observed from years 2008/09 to 2016/17, indicating a growing trend of people interested in meat-replacement products, possibly due to changes in knowledge, awareness, mycoprotein-based foods variety and availability, and/or marketing). This goes in line with the increase in flexitarian (primarily but not strictly vegetarian) people over the last years[42]. The demographic profile of mycoprotein consumers was more likely to be women, white, younger, living in England, belong to a higher socio-economic status, have greater dietary quality scores and less likely to be current smokers relative to non-consumers. This is in line with studies showing that women consume on average less red meat compared to men[43] and that a higher socio-economic status is related to a healthier consumer bias. However, this limited the demographic representativeness of our associations, as we may have not had sufficient data from other demographic groups to explore associations between mycoprotein intake and nutrient, diet quality intake, and NCDs' risk markers.

Flexitarian diets have been shown to be linked to the improvement of a wide range of health outcomes and this goes in hand with the increasing evidence that red and processed meat have a negative impact to both planet and human health. A total of 28.7% of mycoprotein-based foods consumers were self-classed as vegetarian or vegan, it is likely that a considerable proportion of consumers were flexitarian too. Mycoprotein-based foods seem to be an attractive alternative to aid the switch from animal-based protein to plant-based protein, as qualities such as meat-resembling taste and protein bioavailability are not compromised. Furthermore, as we have shown, mycoprotein is positively associated with higher diet quality scores, and lower NCD's risk factors. Therefore, we believe it is paramount to find ways to increase the acceptability of mycoprotein-based foods within demographic groups which are at a particularly higher risk of developing NCDs (i.e. males, older individuals, Black, Asian or minority ethnicities) and have a lower tendency to consume mycoprotein. Of importance, these demographic groups constitute a large proportion of the population at risk of NCD's and could obtain a potential benefit from mycoprotein-based foods. The reasons why there is a smaller representation of other demographic subgroups within the mycoprotein consumer group should be explored, although factors such as economic power, and education could be

involved. It has been reported that higher cost of healthy diets is related to the socioeconomical differences in diet quality[44], and that non-white ethnicities and lower socioeconomical spend on average less pence per person per week (ppw) in meat and vegetables compared to white and higher socioeconomical statuses[45]. In general, mycoprotein-based products have matching costs (£5.67-£6.67/kg) to their animal-derived protein equivalents (£3.30-£8/kg) (UK retailer price). 7 portions (75g each a day) of mycoprotein-based foods a week would represent an approximate cost 297 pence per week (ppw), which is within the average meat expenditure of non-white communities (299-303 ppw), suggesting that the cost of mycoprotein products in the UK may not be the reason why non-white ethnicities are not consuming mycoprotein-based foods in comparison to white communities. For this reason, we think that finding ways to promote mycoprotein-based food within other demographic subgroups may potentially benefit healthy dietary intakes and decrease NCDs' risk at a population level.

This study has some intrinsic limitations derived from its cross-sectional design, such as the inability to infer cause-and-effect relationships as it would have been possible with longitudinal data (including measurements of the same individual over time). Also, NDNS diet data comes from self-reported 4-day food diaries, which are well-known to not be an accurate measurement of food intake. Furthermore, even though we identified some significant associations with NCD's markers and diet quality, we were not able to adjust for physical activity which may act as a potential confounder, as the NDNS database lacked a standard physical activity measurement for all survey years. Importantly, there was a substantial lack of data for some variables, specifically for blood markers and blood pressure, which is likely due to a refusal to have the nurse visit or due to unobtained results following blood sample analysis. These variables possessed almost 50% of missing data for both consumer and non-consumer group. Post-hoc analysis showed some statistically significant differences in nutrient intakes and proportion of ethnic groups between participants with and without missing data for blood markers, BMI and blood pressure, particularly for the non-consumer group. Consequently, the results of the present analysis may be biased as they fail to capture the whole cohort, also potentially leading to loss of power for some variables.

In conclusion, 3.44% of the surveyed population were identified as mycoprotein consumers, and these were likely to be women, white, younger, living in England, belong to a higher socio-economic status, and less likely to be current smokers relative to non-consumers. Mycoprotein consumers had on average a higher intake of dietary fibre, total energy, DASH

and HDI scores and lower BMI compared to non-consumers. There was a positive association with mycoprotein consumers and HDI, DASH score, fibre, total and food energy intake, but a negative association with energy density, sugars, alcohol intake, and FBG and HbA1c NCDs' risk markers. This suggests that mycoprotein-based foods can have a positive effect at reducing NCDs's risk and efforts should be made to promote mycoprotein-based foods consumption within demographic groups at risk of NCDs's. Further investigations are needed to contrast these findings using more complete databases or longitudinal studies.

Conflict of Interest

A.C.M is funded by a non-string educational grant from Quorn Foods Ltd. G.S.F. is currently giving and has given consultant advice to Quorn Foods Ltd.

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Authorship

The authors' contributions are as follows: A.C-M formulated the research question, designed the study, obtained the data from the UK data archive, preprocessed, analysed, interpreted the data and wrote the article; G. S. F. supervised the study and contributed to the writing of the discussion. All authors approved the final version of the manuscript.

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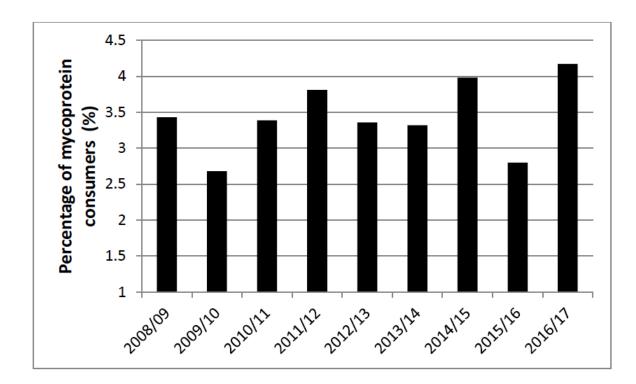
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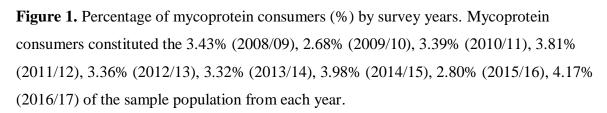
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Figure 1. Percentage of mycoprotein consumers (%) by survey years. Mycoprotein consumers constituted the 3.43% (2008/09), 2.68% (2009/10), 3.39% (2010/11), 3.81% (2011/12), 3.36% (2012/13), 3.32% (2013/14), 3.98% (2014/15), 2.80% (2015/16), 4.17% (2016/17) of the sample population from each year.

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Table 1. Nutritional composition of mycoprotein, Quorn and beef mince.

	Mycoprotein (dry weight)[1]	Mycoprotein (wet weight, ww)[2]	Quorn mince (ww)		Beef mince, 5% fat (ww)
	Per 100g	Per 100g	Per 100g	Per 75g (serving)	Per 100g (serving)
Energy (kcal)	340	85	92	69	168
Protein (g)	44	11	13	9.9	31.3
Carbohydrate (sugars) (g)	36 (0)	9 (0)	2.3 (0.1)	1.7 (0.1)	<0.5(<0.5)
Fat (saturates) (g)	12 (2.8)	3 (0.7)	1.7 (0.5)	1.2 (0.3)	4.7 (2.1)
Fibre (AOAC) (g)	24	6	7.5	5.6	<0.5

Source[1]: Finnigan et al., 2017

Source[2]: https://www.quornnutrition.com/importance-of-micronutrients

Source[3]: Sainsbury's website

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Table 2. Characteristics of mycoprotein-based foods consumers compared to non-consumers in the UK adult population (>18 years old) based on NDNS years 2008/09-2016/17 (N=5507)

Only adults		Mycoprotein-based foods consumers n=191	Non-mycoprotein consumers n=5316	P-value
Mycoprotein kcal	Median	127.4	-	
	IQR	151.37	-	
Mycoprotein (g)	Median	112.48		
	IQR	132.88	-	
Total energy from mycoprotein (%)	Median	7.84	-	
	IQR	8.91	-	
Age	Median	42	49	<0.001
	IQR	20	28	
Sex (%)	Male	38	42	0.24
	Female	62	58	
Ethnicity (%)	White	95	92	0.42
	Mixed ethnic	1	0.8	
	Black or Black British	1	2.1	
	Asian or Asian British	2.6	3.4	
	Any other group	0.5	1.3	
Region (%)	England	67	59	0.18
	Scotland	12	14	
	Wales	15	14	
	Northern Ireland	3.1	5.4	
Socio-economic statues (%)	Higher managerial and professional occupations	22	15.7	0.12
	Lower managerial and professional occupations	30	23.8	
	Intermediate occupations	12	9.9	
	Small employers and own account workers	8.4	10.7	
	Lower supervisory and technical occupations	7.3	9	
	Semi-routine	9.4	14.4	

	occupations			
	Routine occupations	6.2	12	
	Never worked	0.5	0.3	
	Others	3.7	0.04	
Smoking status (%)	Current smoker	13	22	0.02
	Ex-regular smoker	26	25	
	Never regular smoker	61	53	
Dietary fibre intake (g/day)	Median	23	17	<0.001
	IQR	9.16	8.69	
Alcohol intake (g/day)	Median	3.09	0.84	0.21
	IQR	12.95	16.62	
Energy intake (kcal/day)	Median	1809.07	1692.73	0.53
	IQR	726.85	711.50	
DASH score	Median	2	1	<0.001
	IQR	1	1	
HDI	Median	6	6	<0.001
	IQR	1	1	

IQR, interquartile range; DASH, dietary approaches to stop hypertension; HDI, healthy diet index.

Survey-adjusted generalised linear model was used to investigate the association between being a mycoprotein consumer and demographic variables. No variables were used to adjust the model.

P-value ≤0.05 considered to be a significant association.

Table 3. Nutrient values in UK adults (≥18 years) based on NDNS 2008/09-2016/17, in consumers of mycoprotein-based foods and non-consumers (N=5507) and association of mycoprotein consumers and nutrients.

Nutrients (percentage of total energy, kcal)	Consumers n=191		Non-consumers n=5316		% difference vs. non-consumers	P-value	Association between mycoprotein consumers and nutrients		
	Survey adjusted mean	SE	Survey adjusted mean	SE			Beta	SE	P-value
Food energy (kcal)	1792.9	50.32	1741.7	10.26	2.86	0.32	0.22	0.1	0.001
Energy (kcal)	1861.8	53.72	1832.3	11.09	1.58	0.63	3.09	0.5	0.001
Food energy density (kcal/g)	0.63	0.02	0.66	0.001	-4.76	>0.99	-0.1	0	0.04
Protein	15.39	0.35	16.73	0.08	-8.71	0.001	-0	0	0.75
Fat	33.56	0.55	33.28	0.12	0.83	0.62	0.76	0.6	0.19
Saturated fatty acids	12.4	0.35	12.31	0.07	0.73	0.8	0.04	0.4	0.89
CMUFA	11.87	0.25	12.11	0.06	-2.02	0.36	-0.1	0.3	0.63
CN6PUFA	5.11	0.16	4.78	0.03	6.46	0.04	0.57	0.2	0.001
CN3PUFA	1.04	0.04	0.98	0.01	5.77	>0.99	0.1	0	0.01
Trans fatty acids	0.56	0.03	0.54	0.01	3.57	>0.99	0.04	0	0.35
Carbohydrate	47.67	0.6	45.55	0.16	4.45	<0.001	0.21	0.6	0.73
Total Sugars	19.6	0.51	19.32	0.12	1.43	0.59	-1.8	0.6	0.001
Starch	28.04	0.58	26.2	0.14	6.56	0.001	1.97	0.5	0.001
Non milk extrinsic sugars	11.35	0.52	11.7	0.13	-3.08	0.51	-3.2	0.6	0.001
Free Sugars	11.31	0.5	11.37	0.12	-0.53	0.91	-2.8	0.5	0.001
Alcohol (g)	9.84	1.41	12.95	0.49	-31.61	0.04	-1.4	0.2	0.001
Fibre (g)	23.94	0.7	18.63	0.17	22.18	<0.001	3.17	0.5	0.001
HDI	6.57	0.11	5.98	0.02	8.98	<0.001	0.19	0	0.001
DASH score	1.8	0.1	1.38	0.02	23.33	<0.001	0.26	0.1	0.001

CMUFA, monounsaturated fatty acids; CN6PUFA, CN6 poly unsaturated fatty acids; CN3PUFA, CN3 poly unsaturated fatty acids; HDI, healthy diet index; DASH, Dietary approaches to stop hypertension score; SE, standard error of the mean. Differences between consumers and non-consumers were tested using independent t tests assuming unequal variance. P-value ≤0.05 considered to be a statistically significant difference between consumers and non-consumers. Associations were obtained by performing a survey-adjusted generalised linear model with predictors: age, sex, BMI, ethnicity, region of residency, socio-economic and smoking status, alcohol, energy density, total energy, HDI, fibre (expect mycoprotein fibre). Energy density and energy intake were used for all measurements except for energy density (where energy density as predictor was omitted), energy (where energy intake as predictor was omitted), alcohol (where alcohol as a predictor was omitted), fibre (where fibre (except mycoprotein fibre) as a predictor was omitted), and HDI (where HDI as a predictor was omitted).

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Table 4 NCDs' risk markers values in consumers of mycoprotein based foods and non-consumers in UK adults (≥18 years) based on NDNS 2008/09–2016/17, and association of mycoprotein consumers and NCD's risk markers.											
				Non-consumers					**Association between mycoprotein consumers and nutrients**		
NCDs' risk marker	Survey adjusted mean	SE	n	Survey adjusted mean	SE	n	% difference vs. non-consumers	P-value			
									Beta	SE	P-value
BMI (kg/m2)	26.23	0.42	176	27.48	0.11	5069	-4.77	0	-0.8	0.41	0.05
SBP (mmHg)	122.75	1.71	119	125.31	0.41	3446	-2.09	0.15	-0.36	1.86	0.84
DBP (mmHg)	72.18	1.13	119	72.93	0.27	3446	-1.04	0.52	-0.16	1.25	0.89
TC(mmol/l)	4.97	0.13	94	4.94	0.03	2843	0.60	0.82	0.09	0.13	0.48
TAG(mmol/l)	1.25	0.09	94	1.25	0.02	2835	0.00	>0.99	0.01	0.09	0.94
HDL-C(mmol/l)	1.45	0.05	94	1.41	0.01	2843	2.76	>0.99	0.02	0.05	0.72
LDL-C(mmol/l)	2.98	0.11	94	2.98	0.02	2812	0.00	>0.99	0.06	0.11	0.61
TC:HDL-C	3.6	0.15	94	3.72	0.03	2843	-3.33	0.43	-0.04	0.13	0.76
CRP(mg/l)	3.65	0.74	95	3.67	0.15	2843	-0.55	0.98	-0.05	0.86	0.95
FBG (mmol/L)	4.97	0.08	83	5.38	0.03	2716	-8.25	>0.99	-0.31	0.1	0.001
HbA1c (%)	5.35	0.05	90	5.59	0.02	2754	-4.49	>0.99	-0.15	0.06	0.01

BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TAG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; CRP, C-reactive protein; FBG, fasting blood glucose; HbA1c, glycated haemoglobin A1c; SE, standard error of the mean; n, sample size. Differences between consumers and non-consumers were tested using independent t tests, assuming unequal variance. P-value ≤0.05 considered to be statistically significant difference between consumers and non-consumers. Associations were obtained by performing a survey-adjusted generalised linear model with predictors: age, sex, BMI, ethnicity, region of residency, socio-economic and smoking status, alcohol, energy density, total energy, HDI, fibre (expect mycoprotein fibre).
For BMI, BMI as a predictor was omitted.