A randomized controlled trial in young women of the effects of consuming pork meat or iron supplements on nutritional status and feeling of wellbeing

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Report prepared for the Co-operative Research Centre for an Internationally Competitive Pork Industry

By

Jennifer O McArthur [1], Peter Petocz [2], Ian D Caterson [1,3] and Samir Samman [1*]

[1] Discipline of Nutrition and Metabolism, School of Molecular Bioscience, University of Sydney, NSW 2006

[2] Department of Statistics, Macquarie University, Ryde NSW 2113

[3] Boden Institute of Obesity, Nutrition and Exercise, University of Sydney, NSW 2006 Australia

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Executive Summary

Limited information is available on the role of pork meat in influencing iron status. The aim of the present study is to determine if an increase in the intake of pork meat over a period of 3 months will impact the nutritional status of iron, zinc, vitamin B6, vitamin B12, and the feeling of wellbeing in young women.

Young women were randomly assigned to a control diet (CG), a pork containing diet (PG) or a control diet with iron supplementation (SG) for 12 weeks.

Sixty-five women aged 24.6 ± 4.4 y (mean ± SD) completed the trial. Serum ferritin concentrations were increased significantly ($P=0.001$) in subjects assigned to SG as compared to the other groups, as assessed by repeated-measures ANOVA. At week 12, haemoglobin concentrations were significantly higher in PG and SG as compared to individuals in CG. Plasma zinc concentrations at the end of the intervention were similar to baseline concentrations for individuals in the CG and PG but were decreased significantly ($P<0.05$) in SG. Plasma-, erythrocyte-folate, serum vitamin B6 and serum vitamin B12 concentrations were not significantly affected by the intervention, although the concentrations of vitamins B6 and B12 tended to increase in PG. Responses to the Health Survey Short Form (SF36) showed a significant improvement in the scores for “vitality” in SG as compared to subjects assigned to CG or PG. In those consuming pork, the score for “bodily pain” was more favourable than scores in CG and SG. No significant relationships were observed between health concept scores and biomarkers of nutritional status.

Consumption of pork meat by young women maintains haemoglobin levels to the same extent as low-dose iron supplementation, and enhances the feeling of wellbeing.

Key words: Iron, zinc, vitamin B6, vitamin B12, folate, pork, meat, iron supplement, wellbeing

Short title: Effect of pork meat and iron

Table of Contents

Executive Summary i

1. Introduction 1

2. Methodology 2

3. Outcomes 4

4. Application of Research 11

5. Conclusion 11

6. Limitations/Risks 13

7. Recommendations 13

8. Acknowledgements 14

9. References 15

Appendix 1: Health and Well Being Questionnaire 18

1. Introduction

The World Health Organization has estimated that iron deficiency is one of the most prevalent nutrient deficiencies in the world affecting an estimated two billion people [1]. Iron deficiency may occur at any stage of the lifecycle however, children, adolescents, pregnant and postpartum women are commonly affected because of the high iron requirements of growth, pregnancy and lactation. The most common symptom of iron deficiency is anaemia, which, in its severest form, is associated with weakness and eventually heart failure [1-3].

Iron status of men and women has been shown to be lower in those consuming a vegetarian diet as compared to those eating an omnivorous diet [4,5]. A low intake of heme iron and presence of factors that inhibit non-heme iron absorption are the most likely contributors to the impairment of iron absorption in those consuming vegetarian diets [6]. The extent of iron deficiency, especially in young women can be exacerbated in athletic populations [7] mainly due to poor diets and a high metabolic turnover rate of body iron.

Iron status of adolescents was assessed in a national survey of Australian schools. Iron status was considered generally satisfactory except in 15 year old girls where the percentage of iron deficiency was markedly higher (9.2%) compared to younger girls or boys (0-1.6%). There were minimal differences in the quantities of food that were consumed from the "meat group" between the 10- and 15- year old girls [8] suggesting that although iron requirements had increased greatly among adolescent girls, their diets changed little. In a survey of young females, the incidence of iron deficiency and iron deficiency anaemia was observed to be similarly high (7.2 and 4.5%, respectively). A large proportion of the study participants classified themselves as vegetarians (13%), reported high menstrual scores, and consumed small amounts of red meat [9]. In a sample of New Zealand women, mild iron deficiency was observed in 23% of the cohort with risk factors identified as a low intake of meat, fish and poultry; high menstrual blood loss and recent blood donation [10]. Thus the combination of increased iron requirement for growth, iron loss through bleeding, and poor food selection, increase the risk of iron deficiency in women [8-11].

Iron deficiency is associated with decreased general health and increased fatigue. When iron deficient women were supplemented with iron or advised to consume a diet high in iron, particularly a diet high in beef or lamb, their iron status tended to improve [12,13] and their levels of fatigue declined [14]. The role of other types of meat, especially pork, in addressing iron status has been largely unexplored. Pork meat contains a number of essential nutrients including iron, zinc, vitamins B12 and B6. A recent analysis of pork meat identified it also as a source of folate [15].

In short term studies, meals that contain pork meat have been shown to increase the fractional absorption of iron and consequently increase the net amount of iron absorbed by women [16, 17]. Young healthy Danish women were assigned to either a control meal that contained rice, tomato, peas and bread, or the control meal which has added 3 levels of pork meat: 25, 50 and 75g. Iron absorption was determined by radioisotopic methods. It was shown that 25g of pork meat did not increase non-heme iron absorption, whereas absorption increased significantly by 2.6% and 3.4%, respectively, when 50 and 75 g meat were added to the meal [17]. This corresponds to an absorption rate that was 2.6% and 3.4% higher, respectively, than that with the control meal.

The aim of the present study is to determine if an increase in the intake of pork meat over a period of 3 months will impact the nutritional status of iron, zinc, vitamin B6, vitamin B12, and the feeling of wellbeing in young women.

2. Methodology

Women were recruited between August 2008 and July 2009 through advertising and leaflet distribution on the University of Sydney campus. Potential volunteers were screened by using a short questionnaire, and selected based on age (18-35y). Exclusion criteria included vegetarians, those who were pregnant, lactating, or reported a major illness (e.g. gastrointestinal disease), and those consuming nutritional supplements or medication. The University of Sydney Human Ethics Review Committee approved the study and all subjects gave written informed consent prior to their participation.

The study had a parallel design with subjects randomly assigned, by using computer-generated random numbers, to one of 3 groups: control, pork meat diet intervention or supplement intervention. Subjects that were assigned to the control group (CG) were asked to continue with their existing eating patterns and were provided with general dietary advice. They also consumed a placebo capsule that contained 220 mg cellulose (see below). Participants allocated to the pork diet group (PG) were asked to incorporate pork meat into 3 of their meals each week. The meat was provided to the volunteers as part of their participation in the study, and they were asked also to consume a placebo capsule. Participants in the iron supplement group (SG) were provided with the general dietary advice, but were given an iron-containing supplement that contained 37.4 mg elemental iron as 300 mg ferrous gluconate and 200mg of ascorbic acid. The active and placebo supplements were physically identical, and were prepared specifically for the trial by a compounding pharmacist (Royal Prince Alfred Medical Centre Pharmacy, NSW).

Subjects were asked to follow the dietary advice and the supplementation regimen for 12 weeks. Seventy-six women met the selection criteria and were randomly assigned to treatment (Figure 1). All participants had individual consultations with a dietitian on a monthly basis. Height was measured once, to the nearest 0.1cm (Harpenden Stadiometer, Holtain Ltd, Crymch, UK). At monthly visits, the subjects were weighed, in light clothing and no shoes, to the nearest 0.1kg on an electronic scale (Tanita, Corporation, Kewdale, USA). Participants in PG were instructed how to incorporate pork meat into their diets. At 2 weekly intervals, participants were provided with 1.0 kg of frozen, uncooked pork. A variety of pork meat was provided, and the choice of meats was customized based on preferences expressed by each participant. Participants in SG and CG were provided at each dietetic review with varied food packs that contained a range of foods including small portions of ready-to-eat cereals and dairy. At monthly intervals all subjects were provided with a known number of capsules and asked to return any unused ones. These were counted to determine apparent compliance.

Blood samples were collected from all volunteers at the start of the intervention (week 0) then at 4 weekly intervals (weeks 4, 8 and 12). All samples were taken between 0730 and 0930 h from an antecubital vein. Subjects were in a fasted state (10-12 h) and in the supine position during the blood collection. On each occasion, blood samples were collected into vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ, USA): EDTA-coated tubes for the subsequent analysis of erythrocyte folate, haemoglobin, plasma vitamin B6, and plasma zinc concentrations, and untreated tubes for the analysis of iron biomarkers (serum iron, ferritin, transferrin), serum folate and vitamin B12 concentrations. Samples for the analysis of vitamin B6 were collected in tubes that were shielded from light. Blood samples were kept on ice for up to 2h, and centrifuged at 1,500g for 10 min at 5°C.

The concentrations of vitamin B12, serum folate, erythrocyte folate, and ferritin were determined using an automated system (UniCel DxI Immunoassay System, Beckman Coulter Inc. Ca, USA). Serum iron concentrations were determined by a colorimetric method (Cobas Fara II, Hoffman-La Roche Inc, NJ, USA). Transferrin concentrations were determined by an automated method (Immage, Beckman Coulter Inc. Ca, USA), and

Transferrin saturation was determined by dividing the serum iron concentration by the Total Iron Binding Capacity. Plasma zinc concentrations were determined using atomic absorption spectrometry (SpectrAA 20 plus, Varian Inc, Melbourne, Australia). Plasma vitamin B6 (pyridoxal-5-phosphate) concentrations were determined using an HPLC method (Chromsystems Instruments & Chemicals GmbH, Munich, Germany).

Habitual dietary intake was assessed using a semi-quantitative Food Frequency Questionnaire (FFQ) that had been validated for use in a similar population group [18]. Portion sizes in the FFQ included metric sizes and standard size descriptions. The FFQ contained nine frequencies: never, less than 1 per month, 1-3 per month, 1 per week, 2-4 per week, 5-6 per week, 1 per day, 2-3 per day and more than 4 per day. The nutrient contribution of each food was determined by multiplying the frequency value from the FFQ by the serving size of each food item. The average daily nutrient intake was calculated with a purpose-built program (Microsoft Access 2007) linked to a database of Australian food composition (NUTTAB 2006) [19]. Participants at the beginning and conclusion of the trial completed the FFQ. Recall was over the past three months and the questionnaire took approximately 45 minutes to complete. Adherence with the intervention was determined at 4 weekly intervals by use of a 7-day food diary. Studies routinely use 3–12months as their reference period. We set the reference period for this FFQ to match the intervention period of 12 weeks. The FFQ used for this study was based on the original Harvard University FFQ and used subsequently by the Blue Mountains Eye Study. The language was adapted to suit the Australian food supply and vernacular by Australian dietitians and has been used with cohorts similar to this study in recent years [18].

The volunteers' feeling of wellbeing was determined by questionnaire [20] before their participation and on completion of the study. The assessment of wellbeing has been in the past inferred from observable behaviour only eg by performing a routine medical examination. In the Health Survey Short Form 36 (SF–36) Health Survey, a definition incorporating the perceptions of the individual is used i.e. inclusion of the frequency and intensity of feeling states including general mental health. The survey also reflects the preferences of the individual so there are inclusions for personal evaluation of their current health status, susceptibility to illness and health outlook. The SF36 measures 9 health concepts that include physical functioning (10 items), social functioning (2 items), role limitations due to physical problems (4 items), role limitations due to emotional problems (3 items), mental health (5 items), energy and vitality (4 items), bodily pain (2 items), general perception of health (5 items), and the perceived change in health over the past 12 months (1 item). Respondents are asked to rate the frequency of occurrence of each symptom using a 3-6 level response scale before participating, and on completion of the study. For each health concept, responses were coded and transformed to a score ranging from 0 (worst health state) up to 100 (best health state). A copy of the questionnaire is shown in Appendix 1.

Differences between groups were assessed using repeated-measures analysis of variance with the four values of each variable (at time 0, 4, 8, and 12 weeks) as the response and group as the between-subjects factor. We also carried out secondary analyses with the final (12-week) value as response, group as a fixed factor, and the initial value of the variable and an overall measure of change in health concepts as a covariate. Two overall measures of change in health concepts were utilized: the first consisted of an average of the changes on the sub-measures of well being, adjusted so that higher scores had positive meaning on each; the alternative was a linear combination of five of the eight sub-measures, identified by a factor analysis as the first principal component. As well as the univariate analyses, a multivariate analysis was used to investigate group differences between the whole group of biomarker variables. The package PASW version 18 (previously known as SPSS, SPSS Inc, Chicago, Illinois 2010) was utilized for all statistical analyses. Significance was set at $p < 0.05$. Values are reported as means ± SD unless otherwise stated.

3. Outcomes

Of the 76 subjects who were enrolled in the trial, 11 withdrew mostly due to the inconvenience of being involved in a trial or due to discomfort associated with ingesting a capsule. The withdrawals were mainly in the control (CG, n=6) and iron supplementation (SG, n=4) groups (Figure 1). Sixty-five subjects completed the intervention (CG, n=22; PG, n=21; SG, n=22). At baseline there were no significant differences between the groups in anthropometric measures or in nutrient intakes (Table 1).

Figure 1 - Subject flowchart through the study

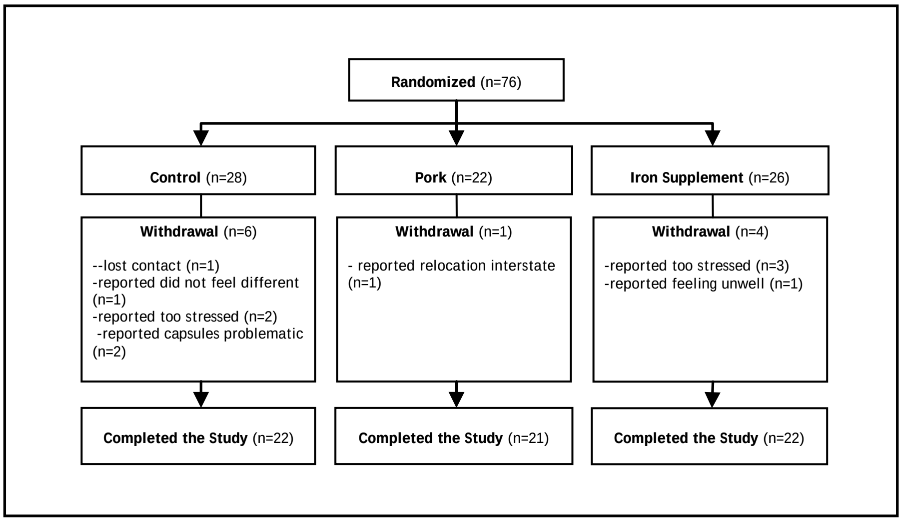


Table 1 - Anthropometric characteristics and dietary intakes of subjects in all intervention groups

Variables	All		CG		PG		SG	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Age (years)	24.6 ± 4.4		25.2 ± 4.2		24.2 ± 4.5		24.6 ±4.6	
Weight (kg)	59.5 ± 8.9	59.5 ± 8.9	61.5 ± 9.2	61.3 ± 8.9	61.6 ± 11.8	62.1 ± 11.4	55.3 ± 5.8	55.1 ± 5.9
BMI (kg/m^2)	21.8 ± 2.8	21.8 ± 2.8	21.8 ± 2.2	21.8 ± 2.1	22.5 ± 3.8	22.7 ± 3.7	21.0 ± 1.9	21.0 ± 2.0
Energy (MJ)	12.7 ± 5.4	11.2 ± 4.9	13.2 ± 5.2	11.3 ± 5.6	14.9 ± 6.9	12.7 ± 5.5	10.7 ±3.6	9.8 ± 3.1
Protein (% en)	20.2 ±3.9	20.5 ± 3.2	21.0 ± 5.0	20.3 ± 3.7	19.6 ± 3.1	21.8 ± 3.4	19.9 ± 3.3	19.8 ± 2.3
Carbohydrate (% en)	44.4 ±3.9	43.2 ±5.6	42.4 ± 7.4	43.4 ± 6.7	44.0 ± 5.4	41.2 ± 5.3	46.6 ± 6.5	44.4 ± 4.4
Fat (% en)	32.9 ±6.1	33.8 ± 4.6	32.9± 6.2	32.9 ± 5.2	34.4 ± 5.9	35.1 ± 4.7	31.8 ± 6.2	33.9 ± 3.8
Alcohol (% en)	1.8 ± 2.8	1.8 ± 2.6	2.9 ± 4.0	2.6 ± 3.9	1.3 ± 1.3	1.3 ± 1.2	1.1 ±1.4	1.3 ± 1.5
Iron (mg)	19.0 ± 7.4	16.8 ± 7.0	19.3 ± 6.8	15.9 ±6.2	21.8 ± 8.77	19.3 ± 8.6	16.6 ± 6.40	16.0 ± 6.4
Zinc (mg)	20.1 ± 8.9	17.9 ± 7.5	21.2 ± 9.9	17.2 ± 7.8	21.9 ± 9.8	20.9 ±8.2	17.6 ± 7.0	16.3 ± 5.9

Variables	All		CG		PG		SG	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Vitamin B6 (mg)	2.3 ± 1.0	2.0 ± 0.9	2.5 ± 1.0	1.9 ± 0.8	2.7 ± 1.1	2.2 ± 1.1	1.9 ± 0.8	1.9 ± 0.9
Vitamin B12 (ug)	4.0 ± 2.2	3.5 ± 1.7	4.2 ± 2.2	3.2 ± 1.6	4.2 ± 2.1	3.9 ±1.9	3.7 ± 2.3	3.5 ± 1.7
Dietary Folate Equivalents (ug)	636 ± 456	566 ± 402	702 ± 590	633 ± 494	784 ± 423	593 ± 407	461 ± 232	484 ± 283

CG: control group; PG: pork diet group; SG: supplement group.

Individuals in PG increased their weekly intake of pork meat by 524 ± 53 g (uncooked), which provided 5.8 ± 0.6 mg of iron. Pork meat cuts that were preferred by the subjects, in decreasing order, were: mince, sausages, pork neck (scotch fillet), pork leg and medallions (Table 2). The cuts provided were purchased from a range of local butchers and supermarkets. The participants could choose the cuts each fortnight to approximate 500g edible portion per week. Recipes and cooking instruction were provided if required. From the food diary analysis, PG had three main meals and two lighter meals (breakfast / lunch) per week. They exchanged chicken or vegetarian meals for the pork meals however the dietetic reviews suggest towards the end of the study some of the beef / lamb meals had been replaced. This was not intentional but rather a result of being familiar with pork meat and for a notable percentage they preferred the pork for particular dishes.

Table 2: Pork cuts selected by volunteers in the Pork Intake group.

Pork Cuts	% of subjects who selected the cut	Meat provided per 2 weeks (g raw weight, median and range	Iron mg/ 100g raw weight
Mince	85	320 (180 – 1100)	1.2
Neck / Scotch Fillet	70	225 (100 – 1200)	1.2
Fillet	3	310 (268 – 900)	1.1
Thick Sausages (duo packs)	76	400 (200 – 600)	1.0
Medallions	25	185 (113 – 400)	1.0
Butterfly Steaks	2	115 (95 – 200)	0.6
Leg (cubed or strips)	51	200 (100 – 525)	0.9
schnitzel ready for crumbing	21	170 (110 – 250)	0.8
Loin Chop	21	200 (130 – 400)	0.8
Ham	47	250 (100 – 250)	0.9

Despite the increase in the consumption of pork meat, there were no significant changes detected in the volunteers' intakes of protein or iron at 12 weeks. Pork meat was incorporated into the diet in exchange for other meats such as beef, lamb, poultry and fish. Compliance with supplementation based on the number of capsules issued and returned, was similar for all groups with 89%, 90% and 93% for CG, PG and SG, respectively. None of the subjects reported gastrointestinal side effects and body weight did not change in any of the intervention groups.

Iron status was affected to some extent by the interventions (Table 3). Compared to CG and PG, volunteers in SG showed transient increases in serum iron concentrations and transferrin saturation, both increased at Week 8 but returned to baseline or below baseline levels by Week 12; despite these different patterns, the repeated-measures ANOVA did not indicate any significant differences between groups. In contrast, a sustained and significant increase (P=0.001) in serum ferritin concentrations was observed in subjects assigned to SG as compared to the other groups (Table 3), and the percent change from baseline was significantly higher at Week 12 as compared to Week 0 (Figure 2). When haemoglobin concentrations are expressed as the difference from baseline values, the change at Week 12 was significantly (P<0.05) higher in PG and SG as compared to individuals in CG. This was mainly due to blood haemoglobin falling below baseline values in CG whilst the concentrations were maintained in PG and SG. When assessed by repeated-measures ANOVA, no significant differences were seen in haemoglobin concentrations between any of the groups (Table 3).

Table 3 - Iron and zinc status of young women in the control (CG), pork diet (PG) or iron supplement (SG) group

Week of Study	0	4	8	12
Serum Iron (μmol/L)				
CG (n=22)	16.5 ± 6.4	16.8 ± 9.3[1]	16.2 ± 9.4	17.8 ± 8.0
PG (n=21)	19.4 ± 7.3	17.0 ± 5.3	17.2 ± 6.8	17.2 ± 7.7
SG (n=22)	17.9 ± 6.0	20.2 ± 9.2	22.0 ± 9.0	18.2 ± 8.8
Serum Ferritin (μg/L)				
CG (n=22)	29.5 ± 22.4	28.9 ± 21.1[1]	26.1 ± 19.1	30.0 ± 20.4 [a]
PG (n=21)	27.3 ± 17.2	24.8 ± 16.9	23.5 ± 19.4	23.0 ± 15.9 [b]
SG (n=22)	34.5 ± 29.1	41.7 ±30.8	44.5 ± 32.5	45.2 ± 26.6 [a,b]
Hemoglobin (g/L)				
CG (n=22)	130.9 ± 9.1	131.3 ± 10.1[1]	131.6 ± 8.2	127.5 ±8.0 [c,d]
PG (n=21)	132.9 ± 7.3	135.8 ± 6.7	133.1 ± 8.5	132.8 ±7.4 [c]
SG (n=22)	132.2 ± 6.7	134.0 ± 7.2	132.2 ± 7.4[1]	132.6 ± 7.4 [d]
Serum Transferrin (g/L)				
CG (n=22)	3.1 ± 0.6	3.0 ± 0.5[1]	3.0± 0.5	2.9 ±0.5
PG (n=21)	3.0 ± 0.6	2.9 ± 0.4	2.9 ± 0.4	2.8 ± 0.4
SG (n=22)	2.9 ± 0.6	2.7 ± 0.5	2.7 ±0.5	2.6 ± 0.5
Transferrin Saturation (%)				
CG (n=22)	22.0 ± 9.4	23.0 ± 14.4[1]	22.2 ±12.7	25.1 ± 12.1
PG (n=21)	26.4 ± 9.0	23.4 ± 7.7	24.4 ± 10.2	24.4 ± 11.5
SG (n=22)	25.5 ± 9.8	29.0 ± 11.0	33.7 ± 14.6	28.0 ± 12.0
Zinc (μmol/L)				
CG (n=22)	14.0 ± 2.4[2]	14.0 ± 2.1[3]	14.4 ± 2.4[2]	14.4 ±2.8[2] [c,d]
PG (n=21)	15.1 ± 2.9	14.5 ± 2.2	15.1 ±3.0	14.4 ± 2.2 [c]
SG (n=22)	14.9 ± 3.3	13.9 ± 3.9	14.1 ± 3.2	12.8 ± 3.3 [d]

CG: control group; PG: pork diet group; SG: supplement group.

[a,b] Values for different groups sharing a common superscript were significantly different by repeated-measures ANOVA, P<0.001

[c,d] Values sharing a common superscript are significantly different at Week 12, P<0.05

[1] n=21, [2] n=20, [3] n=19

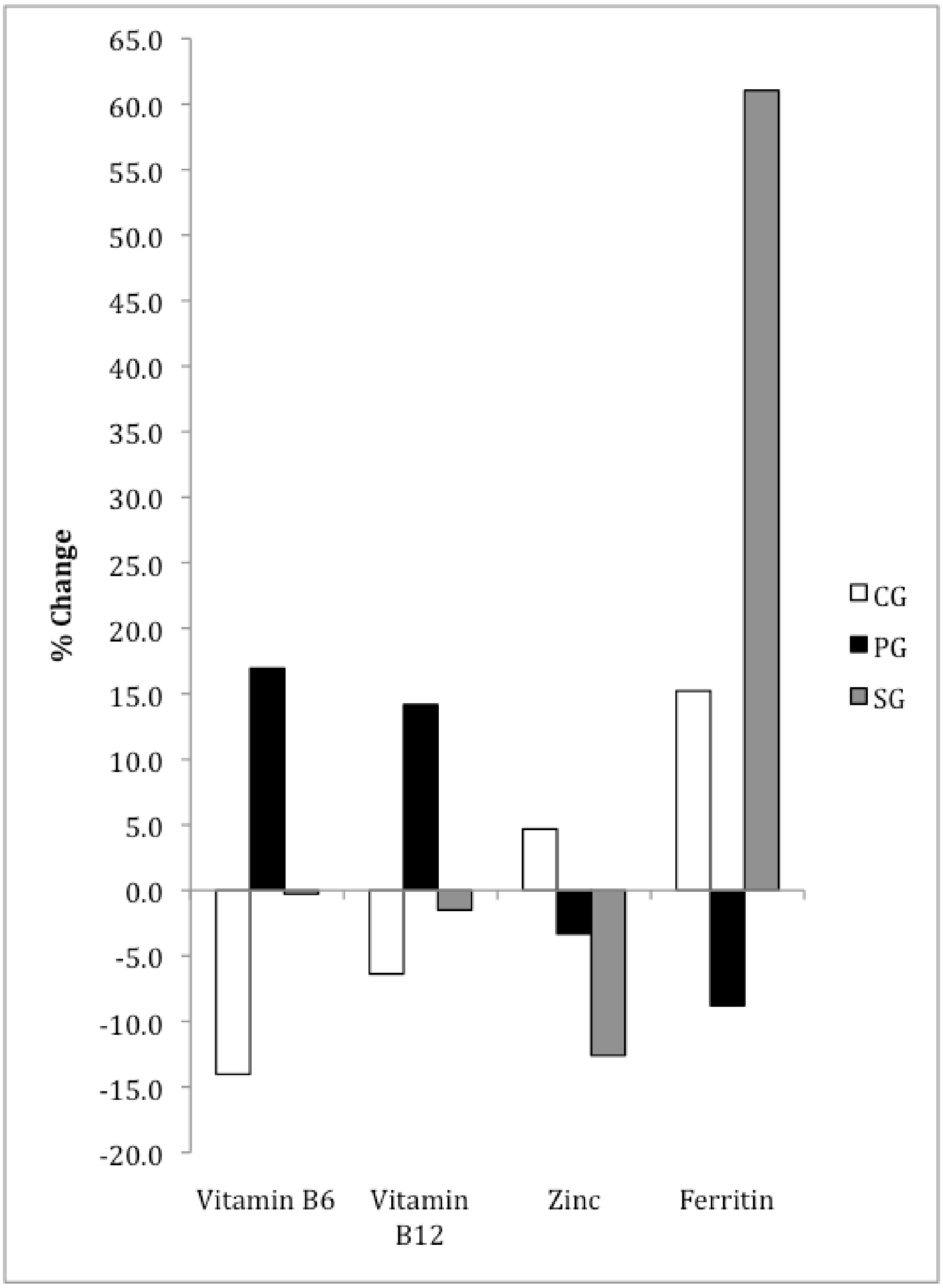
Over the course of the intervention, plasma zinc concentrations showed a marginal decrease (P=0.059 from repeated-measures ANOVA) in SG compared to CG and PG, and were significantly lower (P<0.05) by Week 12 as compared with concentrations recorded at baseline (Table 3). Plasma- and erythrocyte-folate, serum vitamin B6 and serum vitamin B12 concentrations were not significantly affected by the intervention (Table 4), although the change in vitamin B6 and vitamin B12 concentrations at Week 12 showed an upward trend in PG (Figure 2).

Table 4 - Folate, Vitamin B6 and vitamin B12 concentrations in the control (CG), pork diet (PG) or iron supplement (SG) groups

Week of study	0	4	8	12
Vitamin B6 (nmol/L)				
CG (n=22)	115.5 ± 49.6	96.4 ± 24.4[1]	94.5 ±22.9	90.1 ±18.5
PG (n=21)	94.1 ± 26.7	88.8 ± 28.9	90.8 ± 18.0	107.1 ± 72.1
SG (n=22)	134.8 ± 97.2	107.2 ± 27.0	118.7 ± 87.2	110.8 ± 48.0
Vitamin B12 (pmol/L)				
CG (n=22)	258.3 ± 160.4	262.9 ± 147.9[1]	250.3 ± 164.8	244.7 ± 158.0
PG (n=21)	241.0 ± 88.9	241.0 ± 83.1	264.6 ± 94.5	269.2± 102.8
SG (n=22)	286.2 ± 118.9	265.9 ± 110.4	273.5 ± 116.3	279.0 ± 119.8
Serum Folate (nmol/L)				
CG (n=22)	19.6 ± 7.6	20.3 ± 7.1[1]	19.2 ± 7.9	19.0 ± 5.9
PG (n=21)	19.6 ± 9.1	17.6 ± 8.0	18.2 ± 7.1	16.2 ± 7.0
SG (n=22)	26.3 ± 9.6	23.9 ± 10.3	23.8 ± 8.5	23.2 ± 9.2
RBC Folate (nmol/L)				
CG (n=22)	1046.5 ± 1047.4	779.3 ± 302.6[1]	804.6 ± 323.4	814.9 ± 296.6
PG (n=21)	825.0 ± 360.5	740.9 ± 369.9	736.8 ± 374.9	748.6 ± 296.2
SG (n=22)	889.2 ± 337.5	766.4 ± 274.6	843.9 ± 309.5	833.0 ± 232.4

CG: control group; PG: pork diet group; SG: supplement group.
[1] n=21

Figure 2 - Change in nutrient status



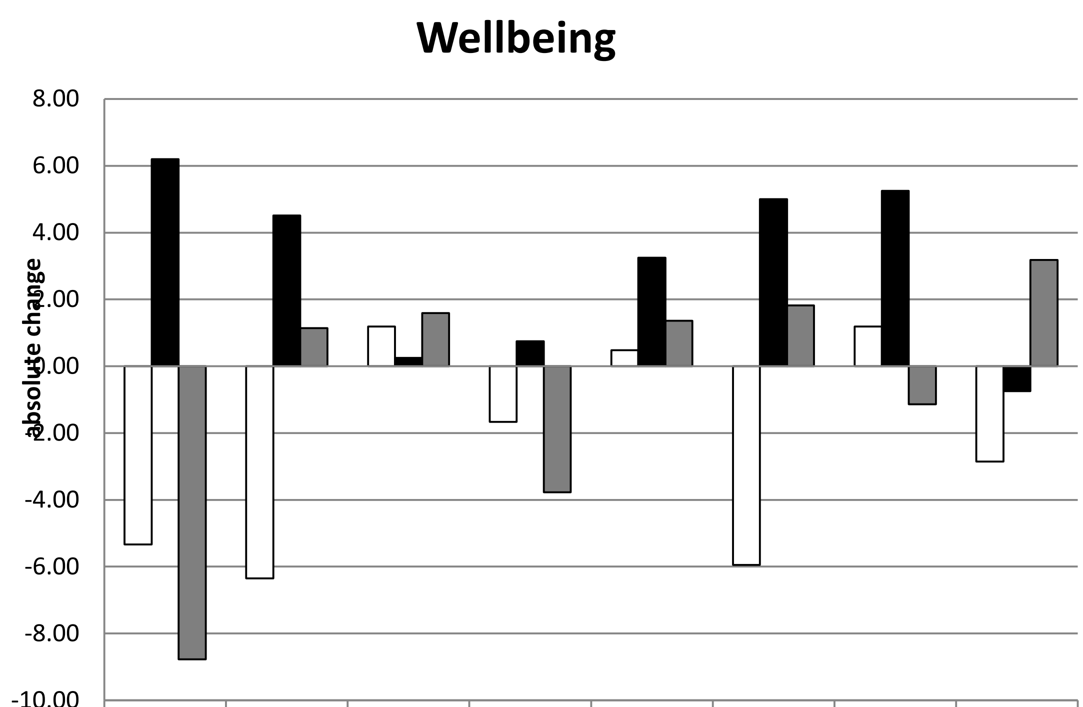
Responses to the Health Survey showed a significant improvement in the scores for "vitality" in subjects following iron supplementation as compared to subjects assigned to CG or PG. In those consuming pork, the score for "bodily pain", which represents the frequency of bodily pains or discomfort, increased from baseline values and were significantly higher (i.e. more favourable) than scores for CG and SG. No significant changes were observed in the scores of other perceptions of health (Table 5) although a trend for improved health concept scores was seen for all parameters in those in PG (Figure 3). No significant relationships were observed between the health concept scores and any of the biomarkers of nutritional status over the course of the intervention.

Table 5 - Health concept scores (SF36) scores of young women in the control (CG), pork diet group (PG) or iron supplement group (SG).

Wellness Components	CG (n=21)	PG (n=20)	SG (n=22)
Bodily Pain			
Baseline	87 ± 12	79 ± 19 [a]	85 ± 12
After intervention	82 ± 13	85 ± 16 [a]	76 ± 20
Role – Emotional			
Baseline	81 ± 31	82 ± 30	82 ± 32
After intervention	75 ± 36	86 ± 23	83 ± 25
General Health			
Baseline	48 ± 9	49 ± 10	46 ± 8
After intervention	49 ± 7	49 ± 10	48 ± 7
Mental Health			
Baseline	63 ± 8	62 ± 9	66 ± 6
After intervention	62 ± 9	62 ± 12	62 ± 7
Physical Functioning			
Baseline	93 ± 19	90 ± 17	93 ± 15
After intervention	93 ± 17	94 ± 16	95 ± 13
Role – Physical			
Baseline	96 ± 9	86 ± 29	83 ± 29
After intervention	90 ± 20	91 ± 20	85 ± 23
Social Functioning			
Baseline	49 ± 5	44 ± 11	50 ± 9
After intervention	51 ± 7	50 ± 14	49 ± 4
Vitality			
Baseline	50 ± 8	49 ± 9	52 ± 14 [b]
After intervention	47 ± 11	49 ± 14	55 ± 11 [b]

CG: control group; PG: pork diet group; SG: supplement group.
[a,b,] Values sharing a common superscript are significantly different P<0.05

Figure 3. Change in wellbeing scores in young women following a control diet (CG), pork consumption (PG) or supplementation with iron (SG).



	T12 - T0 BP	T12-T0 E	T12-T0 GH	T12-T0 MH	T12-T0 PF	T12-T0 RP	T12-T0 S	T12-T0 V
CG	-5.33	-6.35	1.19	-1.67	0.48	-5.95	1.19	-2.86
PG	6.20	4.52	0.25	0.75	3.25	5.00	5.25	-0.75
SG	-8.77	1.14	1.59	-3.77	1.36	1.82	-1.14	3.18

4. Application of Research

The results of this trial show that consumption of pork meat by young women:

- maintains hemoglobin levels to the same extent as low-dose iron supplementation
- enhances the feeling of wellbeing
- tends to increase the status of vitamins B6 and B12.

These outcomes can contribute to promoting the benefits of pork meat in the community, particularly amongst young women. Specifically, the outcomes point to the benefit of pork consumption in a community group at-risk of iron and vitamin B12 deficiencies.

Opportunities uncovered by the research

The dietetic reviews with participants revealed 3 main issues:

1. there is a lack of food knowledge of pork meat. Most participants did not know how to cook it, and needed instructions and recipes. The exposure to pork by a dietitian yielded acceptance and appreciation of the product. This suggests that the promotion of pork meat by health professionals such a dietitian overcomes any health concerns.
2. the dietary patterns of the young women showed a high proportion of take away meals. This suggests that greater availability of pork meat in take away meals may increase its consumption.
3. The delays of recruitment in this trial were exacerbated by the swine epidemic. This highlighted the misconceptions of food safety of pork.

Commercialization/Adoption Strategies

N/A

5. Conclusion

The results of the present study show that the consumption, by young women, of pork meat or a low-dose iron supplement over 12 weeks is able to maintain similar blood haemoglobin concentrations. In both intervention groups, haemoglobin concentrations at Week 12 were maintained at a level higher than observed for the control group. Also our study shows that individuals who consumed additional pork meat or took iron supplements, reported improvements in their perceptions of health, as indicated by favourable scores for "bodily pain" and for "vitality", respectively.

Adequate iron status is maintained when a meat-containing diet is eaten as compared to a diet that is predominantly vegetarian-based [21], or one that contains oily fish [22] as the main source of animal protein. In adolescents, iron status is higher in those who consume meat as compared to those who consume fish or poultry [23] or predominantly cereal-based foods [8]. The presence of animal protein is thought to produce an enhancing effect on the absorption of non-heme iron [6,11]. In the present study those assigned to PG increased their intake of pork meat mainly in exchange for poultry, and their total intake of protein was unchanged.

Two other intervention studies have employed a trial design that is similar to the present study [12,13]. Heath et al [12] instructed women with mild iron deficiency to increase their intake of iron and its absorption enhancers, from a range of food sources, and to decrease the intake of iron absorption inhibitors (e.g. phytate). No significant effect on biomarkers of iron status was observed after 16 weeks, although serum ferritin concentrations tended to be higher. In iron deficient women, Patterson et al [13] showed that adherence to dietary advice that was similar to that provided by Heath et al [12], produced minor increases in serum ferritin and haemoglobin concentrations. In both trials, iron supplements, equivalent to 50 [11] or 105 [12] mg of elemental iron, increased the women's status of iron. Unlike the previous studies, in the present study the dietary advice focused specifically on increasing the consumption of pork meat, with no other dietary instructions regarding the intake of enhancers or inhibitors of iron absorption. Over the course of the 12-week intervention, the haemoglobin concentrations were similar in those who consumed either pork meat or an iron supplement. Plasma ferritin concentrations increased only in those supplemented with iron, at a dosage of 37.4 mg elemental iron (as ferrous gluconate); an amount that is lower than used in the other studies.

The potential mechanisms by which the consumption of pork meat maintains the concentration of haemoglobin to the same extent as seen in SG are not known. The contribution of iron from pork meat was low, 0.8 mg/day, and equivalent to 5% of the total intake of iron. Thus the similarity in blood haemoglobin concentrations between SG and PG cannot be explained by the intake of iron alone. However, it is possible that the combination of micronutrients that is provided by pork meat, such as, zinc, selenium, vitamins B6 and B12 [15], a high dietary folate status at baseline, in addition to iron, could enhance the synthesis of haemoglobin to the same extent as seen in SG. In the present study, the concentrations of vitamins B6 and B12 were increased by approximately 15% from baseline values in PG, and it has been reported by others that pork meat provides bioavailable quantities of selenium [24]. Barriers to the bioavailability of vitamins B6 and B12 tend to be the presence of food matrix, usually as plant cell wall material that retards absorption, or intrinsic factors such as decreased gastric secretion that compromises the absorption of vitamin B12. The increases in the plasma concentrations of vitamins B6 and B12 in PG suggest that these nutrients are readily bioavailable from pork meat.

Ingestion of iron supplements, as a single nutrient or combined with folic acid, is reported to interfere with zinc absorption. By measuring the post-prandial plasma zinc concentration following a bolus dose of zinc, also known as the zinc tolerance test, Solomons & Jacob [25] and Meadows et al [26] showed that iron retards the appearance of zinc in plasma when iron and zinc are co-ingested. No effect of iron is observed when iron is presented in the form of heme iron [25]. Radioisotope studies suggested that the antagonism between the cations occurs when the supplements are co-ingested in a water solution but not when consumed as part of a meal [27]. In the present study, volunteers were instructed to consume the iron supplement with a glass of water prior to consuming a main meal. Fasting plasma zinc concentrations decreased significantly in those supplemented with iron, despite the low dose of iron, and the relatively small increase in the total dietary Fe:Zn ratio following supplementation (1.0 and 3.3 for the Fe:Zn ratio at baseline and following supplementation, respectively). This observation is consistent with the hypothesis put forward by Solomons [28], which states that a ratio of Fe:Zn greater than 2, when the total amount of ionic species is greater than 25 mg, appears to have a measurable effect on the nutritional status of zinc.

In cross sectional surveys in men [29] and in elderly women [30], it has been shown that plasma zinc is positively related to haemoglobin. In pregnant women, plasma zinc is reported to be the strongest predictor of haemoglobin concentrations [31]. In the present study, the observed significant decrease in plasma zinc concentrations in the iron supplemented group may have contributed to a smaller than expected increase in the concentration of haemoglobin.

Iron supplementation was associated with increases in the subjects' scores for vitality. In response to the Health Survey SF36, volunteers in the present study rated their perception of energy and vitality based on being "tired and worn out" (lowest possible score) to "full of pep and energy all the time" (highest possible score). The improvement in the vitality score following iron supplementation is consistent with previous observations that show fatigue to be alleviated by a higher iron status [14, 32]. Iron deficient men [33] and women [34] are reported to have reduced work capacity, and supplementation with iron has been shown to decrease heart rate and energy expenditure that is associated with work [34]. The observation in the present study that the consumption of pork improves the score for bodily pain is novel, and to our knowledge has not been reported previously. The perception of bodily pain was based on "very severe and extremely limiting pain" (lowest score) to "no pain or limitations due to pain" (highest score). Bodily pain has been shown to be low (unfavourable score) in people with diabetes [35], and the score in obese individuals improves following weight loss [36]. Little is known about the impact on the score of specific food items or diets. It is tempting to speculate that the contribution of the nutrients that are associated with pork meat, e.g. vitamins B6 and B12, and rapport with a health professional may have had a positive influence on the women's perception of health.

In summary, the consumption of pork meat by young women maintains haemoglobin levels to the same extent as iron supplementation, and enhances the feeling of wellbeing. Individuals who are at-risk of iron deficiency would benefit from dietary strategies that include the increase in heme iron and avoidance of inhibitors of iron absorption.

6. Limitations/Risks

The inability of the subjects to consume capsules was a limiting factor in the recruitment and retention of volunteers in trial, and their poor knowledge of food and cooking skills restricted the choices of meat cuts that could be used in the intervention. One of the limitations of the present study is the lack of screening of the volunteers for confounders of iron status, such as underlying inflammation [37] and helicobacter pylori infections [38].

7. Recommendations

As a result of the outcomes in this study the following recommendations have been made:

1. Develop nutrition education programs with the assistance of dietitians and home economists to increase awareness of cooking methods for pork, to diffuse food safety as an issue, and to promote the health benefits of pork meat.
2. Work with food service providers to increase the use and availability of pork meat in take away foods for the general public. Work with hospital food service to promote pork meat as a good source of protein and micronutrients.
3. Increase research that aims to strengthen the evidence for the link between pork meat and health outcomes. This includes clinical studies in a range of groups such as young women and elderly people.

4. Support fundamental research that aims to identify biomarkers of zinc status in humans. This nutrient is essential for a range of biological functions but little is known about biomarkers that can be used to assess zinc status. Pork meat is potentially a good source of bioavailable zinc.
5. Increase the nutrient content of pork meat by either selective breeding and/or by modifying animal feeding practices.

8. Acknowledgements

SS and IDC designed research; SS and JOMc conducted research; JOMc, PP and SS analyzed data; SS, JOMc, PP and IDC wrote the paper. SS had primary responsibility for final content. All authors read and approved the final manuscript. The authors thank Kai Lin Ek, Sabine Kademann, Tony McArthur, Jenny Phuyal and Kamrul Zaman for technical assistance.

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Appendix 1: Health and Well Being Questionnaire

MEAT INTAKE AND IRON STATUS IN WOMEN

The University of Sydney

SF – 36 Women's Health and Well Being Questionnaire

This survey asks for your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities.

Thank you for completing this survey!

NAME ……………………………………………

DATE ……………………………………………

For each of the following questions, please mark an ☒ in the **one** box that best describes your answer.

---

1. In general, would you say your health is:

Excellent	Very good	Good	Fair	Poor
▼	▼	▼	▼	▼
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

2. <u>Compared to one year ago</u>, how would you rate your health in general <u>now</u>?

Much better now than one year ago	Somewhat better now than one year ago	About the same as one year ago	Somewhat worse now than one year ago	Much worse now than one year ago
▼	▼	▼	▼	▼
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

3. The following items are about activities you might do during a typical day. Does <u>your health now limit you</u> in these activities? If so, how much?

		Yes, limited a lot	Yes, limited a little	No, not limited at all
a	**Vigorous activities**, such as running, lifting heavy objects, participating in strenuous sports	☐ 1	☐ 2	☐ 3
b	**Moderate activities**, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	☐ 1	☐ 2	☐ 3
c	Lifting or carrying groceries	☐ 1	☐ 2	☐ 3
d	Climbing **several** flights of stairs	☐ 1	☐ 2	☐ 3
e	Climbing **one** flight of stairs	☐ 1	☐ 2	☐ 3
f	Bending, kneeling, or stooping	☐ 1	☐ 2	☐ 3
g	Walking **more than a mile**	☐ 1	☐ 2	☐ 3
h	Walking **several blocks**	☐ 1	☐ 2	☐ 3
i	Walking **one block**	☐ 1	☐ 2	☐ 3
j	Bathing or dressing yourself	☐ 1	☐ 2	☐ 3

4. During the <u>past 4 weeks</u>, have you had any of the following problems with your work or other regular daily activities <u>as a result of your physical health</u>?

		Yes	No
a	Cut down on the <u>amount of time</u> you spent on work or other activities	☐ 1	☐ 2
b	<u>Accomplished less</u> than you would like	☐ 1	☐ 2
c	Were limited in the <u>kind</u> of work or other activities	☐ 1	☐ 2
d	Had <u>difficulty</u> performing the work or other activities (for example, it took extra effort)	☐ 1	☐ 2

5. During the <u>past 4 weeks</u>, have you had any of the following problems with your work or other regular daily activities <u>as a result of any emotional problems</u> (such as feeling depressed or anxious)?

		Yes	No
a	Cut down on the amount of time you spent on work or other activities	☐1	☐2
b	Accomplished less than you would like	☐1	☐2
c	Did work or other activities less carefully than usual	☐1	☐2

6. During the <u>past 4 weeks</u>, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbours, or groups?

Not at all	Slightly	Moderately	Quite a bit	Extremely
☐1	☐2	☐3	☐4	☐5

7. How much <u>bodily</u> pain have you had during the <u>past 4 weeks</u>?

Nil	Very mild	Mild	Moderate	Severe	Very Severe
☐1	☐2	☐3	☐4	☐5	☐6

8. During the <u>past 4 weeks</u>, how much did <u>pain</u> interfere with your normal work (including both work outside the home and housework)?

Not at all	A little bit	Moderately	Quite a bit	Extremely
☐1	☐2	☐3	☐4	☐5

9. These questions are about how you feel and how things have been with you during the <u>past 4 weeks</u>. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the <u>past 4 weeks</u>…

		All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	None of the time
a	Did you feel full of pep?	☐1	☐2	☐3	☐4	☐5	☐6

b	Have you been a very nervous person?	☐1	☐2	☐3	☐4	☐5	☐6
c	Have you felt so down in the dumps that nothing could cheer you up?	☐1	☐2	☐3	☐4	☐5	☐6
d	Have you felt calm and peaceful?	☐1	☐2	☐3	☐4	☐5	☐6
e	Did you have a lot of energy?	☐1	☐2	☐3	☐4	☐5	☐6
f	Have you felt downhearted and blue?	☐1	☐2	☐3	☐4	☐5	☐6
g	Did you feel worn out?	☐1	☐2	☐3	☐4	☐5	☐6
h	Have you been a happy person?	☐1	☐2	☐3	☐4	☐5	☐6
i	Did you feel tired?	☐1	☐2	☐3	☐4	☐5	☐6

10. During the <u>past 4 weeks</u>, how much of the time has your <u>physical health or emotional problems</u> interfered with your social activities (like visiting friends, relatives, etc.)?

All of the time	Most of the time	Some of the time	A little of the time	None of the time
▼	▼	▼	▼	▼
☐1	☐2	☐3	☐4	☐5

11. How TRUE or FALSE is each of the following statements for you?

		Definitely true ▼	Mostly true ▼	Don’t know ▼	Mostly false ▼	Definitely false ▼
a	I seem to get sick a little easier than other people	☐1	☐2	☐3	☐4	☐5
b	I am as healthy as anybody I know	☐1	☐2	☐3	☐4	☐5
c	I expect my health to get worse	☐1	☐2	☐3	☐4	☐5
d	My health is excellent	☐1	☐2	☐3	☐4	☐5

Thank you for completing these questions!