

## The Effect of National Wheatmeal on the Absorption of Calcium

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When McCance & Widdowson [1942] decided to investigate the effect of brown bread on the absorption of calcium and other minerals from the human gut, the Ministry of Food had not yet announced their intention to introduce a wheatmeal of 85% extraction. The experiments of McCance & Widdowson [1942] were carried out on flours of 92% ('brown flour') and of 69% ('white flour') extraction. Although their data, by way of interpolation, permit an approximate estimate of the properties of a flour of 85% extraction, it seemed desirable, when the national wheatmeal of 85% extraction was adopted, to extend the work to this flour. We undertook such an investigation at the suggestion of Dr McCance. The behaviour of Ca only was investigated, this being representative, as well as from the practical point of view probably the most important of the minerals whose absorption is affected by substances of the outer layers of the wheat grain.

### EXPERIMENTS

The experiments were carried out on six volunteers. Their ages and weights are shown in Table 1. They were men of military age who had been put on the register of conscientious objectors and who had originally volunteered for experiments on scabies [see Mellanby, 1941] which were conducted concurrently with the dietary experiments.

Table 1. *Weights and ages of the volunteers*

No.	Age years	Body weight stripped (kg.)		
		At start	After 4 weeks on National Wheatmeal	4-6 weeks later (after 'white' flour regimen)
1	27	66.0	65.4	67.7
2	31	62.8	62.9	63.8
3	30	56.2	56.2	56.9
4	20	60.2	60.8	60.4
5	30	53.8	53.8	54.7
6	23	50.2	52.2	51.8

The dietary and analytical arrangements were essentially the same as those elaborated and described by McCance & Widdowson [1942]. Data concerning one of the consignments of wheatmeal used have been published elsewhere [Krebs & Mellanby, 1942]. The 'white' flour used came from a different grist and was of 75% extraction. Dr Widdowson determined the phytic acid content of samples of the wheatmeal flour and of the bread, and found 140 mg.

phytic acid P/100 g. flour, and 41.4 mg. phytic acid P/100 g. bread. The H<sub>2</sub>O content of the bread varied between 35 and 38%.

An adverse effect on the Ca balance of phytic acid-containing cereals can be expected only if the Ca content of the diet is relatively low and the cereal content relatively high. In choosing the diet the aim was therefore to keep the Ca content near to the minimum human requirement of 0.55 g./day [Leitch, 1936-7] and to include at least 500 g. wheatmeal flour (dry wt.)/day. Preliminary experiments taught us that the Ca content of the usual diet of the volunteers (during June and July 1941) was above 1 g./day, and that very considerable restrictions had to be imposed in order to reduce the Ca content of the food to the minimum requirement of 0.55 g., namely, (1) complete omission of cheese, (2) restriction of the milk to about 100 ml./day (including milk used in cooked dishes), (3) omission of the vegetables and fruits containing more than 50 mg. Ca/100 g. (e.g. spinach, spinach beet, cabbages, broccoli tops, leeks, spring onions, turnips, water cress, rhubarb, raisins, dried figs), (4) omission of tinned fish (containing softened bones). Further restrictions were due to rationing which allowed approximately 200 g. meat (on the plate), 225 g. fat, 225 g. butter and 123 g. bacon/week. With these restrictions the diet contained during August and September 1941 on average 0.55 g. Ca/day, of which 0.12 g. was derived from milk, 0.12 g. from bread, which had not been fortified by the addition of Ca, and the remaining 0.31 g. from other sources, mainly vegetables. This last source of Ca seems to be subject to very considerable seasonal variations. It stayed at the above-mentioned level of about 0.3 g. during August and the first half of September, but fell later in September and during October to about 0.2 g./day. During the later period it was necessary to raise the milk content of the diet in order to maintain the Ca supply of 0.55 g./day.

Of the flour an average of 70 g. (dry) was eaten in the form of cooked dishes, and the rest in the form of bread. To make the large quantity of bread—600-900 g./day—palatable it was necessary to supply an extra jam ration of 1 lb./head/week.

The average total calorie intake was about 4100/day. Of these 2200 came from flour.

Our main experiment, carried out after preliminary trials, consisted of two periods. During the first, lasting 4 weeks from the middle of August to the middle of September 1941, national wheatmeal was the staple food; the second was a control period, lasting 4 weeks (in one case 3 weeks) between the end of September and early November 1941, when 'white' bread was the staple food.

### RESULTS

It will be seen from Tables 2 and 3 that the consumption of national wheatmeal was followed, under the conditions of our experiment, by an

Table 2. *Data relating to the calcium balance of six volunteers*

Volunteer no.	Week ending	Diet	Intake of		Ca in food (g./week)	Ca in excreta (g./week)			Ca balance (g./week)	Average daily Ca	
			Bread (g./week)	Milk (ml./week)		Urine	Faeces	Total		Intake (g.)	Balance (g.)
1	2. ix. 41	Wheatmeal bread	6160	600	4.21	1.56	3.52	5.08	-0.87	0.580	-0.143
	9. ix. 41	"	7080	600	3.93	1.61	3.46	5.07	-1.14		
	16. ix. 41	"	7140	700	4.26	1.53	3.68	5.21	-0.95		
	23. ix. 41	"	6260	600	3.82	1.60	3.27	4.87	-1.05	0.625	-0.022
	14. x. 41	White bread	5850	950	3.52	1.71	2.37	4.08	-0.56		
	21. x. 41	"	5770	1100	4.61	1.73	3.08	4.81	-0.20		
	28. x. 41	"	5070	1400	4.62	1.67	3.02	4.69	-0.07		
4. xi. 41	"	5450	1400	4.74	1.51	3.02	4.53	+0.21			
2	29. viii. 41	Wheatmeal bread	5800	600	3.92	1.52	4.21	5.73	-1.81	0.531	-0.141
	5. ix. 41	"	4990	700	3.58	1.59	2.66	4.25	-0.67		
	12. ix. 41	"	5620	700	3.68	1.70	2.69	4.39	-0.71		
	19. ix. 41	"	5140	700	3.72	2.03	2.43	4.46	-0.74	0.504	-0.054
	17. x. 41	White bread	5050	1050	3.30	1.98	1.75	3.73	-0.43		
	24. x. 41	"	3930	1400	3.67	1.63	2.42	4.05	-0.38		
	31. x. 41	"	4860	1400	3.63	1.64	2.23	3.87	-0.24		
7. xi. 41	"	5600	1400	3.52	2.09	1.90	3.99	-0.47			
3	28. viii. 41	Wheatmeal bread	5108	700	4.13	2.05	2.94	4.99	-0.86	0.622	-0.073
	5. ix. 41	"	5150	700	4.02	2.35	2.83	5.18	-1.17		
	12. ix. 41	"	5270	700	4.46	2.17	2.29	4.46	±0		
	19. ix. 41	"	5301	700	4.80	2.46	2.36	4.82	-0.02	0.574	+0.038
	3. x. 41	White bread	6387	600	3.34	2.27	1.49	3.76	-0.42		
	10. x. 41	"	5813	500	3.65	2.05	1.38	3.43	+0.22		
	17. x. 41	"	5494	1050	4.40	2.44	1.62	4.06	+0.34		
24. x. 41	"	5584	1400	4.67	2.16	1.58	3.74	+0.93			
4	29. viii. 41	Wheatmeal bread	5368	700	4.42	1.86	2.51	4.37	+0.05	0.560	-0.120
	5. ix. 41	"	5178	700	3.67	2.14	2.45	4.59	-0.92		
	12. ix. 41	"	5165	700	4.13	2.11	3.03	5.14	-1.01		
	19. ix. 41	"	4734	600	3.44	2.05	2.86	4.91	-1.47	0.564	-0.038
	17. x. 41	White bread	4108	1050	3.40	1.85	2.74	4.59	-1.19		
	24. x. 41	"	4695	1400	4.40	2.18	2.44	4.62	-0.22		
	31. x. 41	"	4305	1400	4.05	1.59	1.80	3.39	+0.66		
7. xi. 41	"	4518	1400	3.96	1.60	2.67	4.27	-0.31			
5	2. ix. 41	Wheatmeal bread	4980	700	3.86	1.77	2.46	4.23	-0.37	0.516	-0.118
	9. ix. 41	"	5360	700	3.85	1.92	2.80	4.72	-0.87		
	16. ix. 41	"	5240	600	3.62	1.91	2.61	4.52	-0.90		
	23. ix. 41	"	4560	700	3.10	1.79	2.47	4.26	-1.16	0.600	-0.033
	21. x. 41	White bread	4710	1250	3.81	1.63	2.77	4.40	-0.59		
	28. x. 41	"	4730	1400	4.30	1.82	2.78	4.60	-0.30		
	4. xi. 41	"	5900	1400	4.48	1.61	2.67	4.28	+0.2		
6	2. ix. 41	Wheatmeal bread	5121	700	3.60	1.58	2.22	3.80	-0.20	0.491	-0.065
	9. ix. 41	"	5068	700	3.85	1.76	2.37	4.13	-0.28		
	16. ix. 41	"	4400	700	3.34	1.71	2.11	3.82	-0.48		
	23. ix. 41	"	4512	700	2.96	1.74	2.07	3.81	-0.85	0.442	-0.027
	7. x. 41	White bread	3791	700	2.34	1.41	0.97	2.38	-0.04		
	14. x. 41	"	4667	1350	3.10	2.30	1.17	3.37	-0.27		
	21. x. 41	"	4192	1400	3.27	2.00	2.20	4.20	-0.93		
28. x. 41	"	4306	1400	3.65	1.85	1.33	3.18	+0.47			

average Ca loss of 0.110 g./day. When wheatmeal was replaced by 'white' flour the average balance was still slightly negative, viz. 0.023 g., but this is less than one-quarter of the loss during the wheatmeal period. The effect of wheatmeal is clear-cut in every individual case.

These results are in accordance with those of

McCance & Widdowson [1942]. From Table 17 of their paper we calculate the following figures:

Milling standard %	Average daily Ca intake g.	Average daily Ca balance g.
92	0.558	-0.086
69	0.512	-0.018

Table 3. *Effect of national wheatmeal on the average calcium intakes and balances of six men*

(This is a summary of Table 2; the data on national wheatmeal are the average of 24 weeks, those on white flour of 23 weeks.)

	Average daily bread consumption (not counting approximately 80 g. flour eaten in cooked dishes) (g.)	Average daily Ca intake (g.)	Average daily Ca balance (g.)
Milling standard			
National wheatmeal (85 % extraction)	766	0.550	-0.110
White flour (75 % extraction)	707	0.552	-0.023

In these experiments the bread consumption was somewhat lower (about 530 g./day) than in ours (about 730 g./day). This explains why, in our subjects, the Ca loss was greater than in those of McCance & Widdowson, although we used 85 % extraction flour whilst the data of McCance & Widdowson refer to a 92 % extraction flour.

We wish to emphasize that the conditions under which the adverse effect of national wheatmeal on the Ca balance was observed were exceptional ones. They included an average daily intake of 1.7 lb. of bread (requiring an extra jam ration to make it palatable) as well as a deliberate and severe curtailment of the Ca supply. In a preliminary experiment where the average wheatmeal bread intake was about 1 lb./day and the average daily Ca supply 0.94 g., the Ca balance was in equilibrium (Table 4).

Table 4. *Ca balance at higher level of Ca intake and lower level of wheatmeal bread consumption*

(Average of 14 weekly periods; 5 subjects.)

	g.
Average wheatmeal bread intake	456
Average daily Ca intake	0.941
Average daily Ca excretion	0.911
Average daily Ca balance	+0.030

## SUMMARY

1. Six adult male volunteers were kept on a diet containing a large proportion of bread (on average 766 g. wheatmeal bread of 85 % extraction or 707 g. 'white' bread of 75 % extraction) and about 0.55 g. Ca/day. Ca was determined in food, faeces and urine.

2. In every case national wheatmeal had an adverse effect on the Ca balance. On average 0.110 g. Ca was lost daily during the wheatmeal regime whilst 0.023 g. was lost during the white flour regime.

3. The results are in accordance with those obtained by McCance & Widdowson on 69 and 92 % extraction flours, and support the present policy of adding Ca salts to national wheatmeal.

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## Colorimetric Determination of Total, Free and Ester Cholesterol in Tissue Extracts

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Kelsey's method [1939] for the determination of total, free and ester cholesterol was tested in the course of analyses of lipid extracts of various tissues (liver, kidney, heart, adrenals and blood). This method seemed to be particularly useful, because not only cholesterol, but also total fatty acids, and fatty acids derived from neutral fats and cholesteryl esters, could be determined on one sample. Kelsey claimed that the cholesterol obtained by his method, and estimated colorimetrically, is pure. However, in my experience, the cholesterol thus obtained

is contaminated by substances which often considerably influence the colour developed with the Liebermann-Burchard reagents, and make accurate colorimetric reading impossible. Apart from this disturbing factor, the method gave very low results: cholesterol added to lipid mixtures could not be satisfactorily recovered.

After some experiments a technique has been evolved which retains the advantages of Kelsey's method and is essentially a combination of the method of Schoenheimer & Sperry [1934], as modi-