layers for typical birefringence of starch grains under cross-polarized light showed that starch grains were absent in the upper layer, appeared only occasionally in the middle layer, and made up about 80% of the total bodies in the bottom layer. Some large protein bodies and aleurone grains as well as vascular and reticular materials were also present in the bottom layer (Figure 6). The distinction between aleurone grains and protein bodies was made on the basis of the presence or absence of inclusion bodies within the particulates. On this basis, there appeared to be little difference between the particulates of the upper and middle layers. Weights of particulates obtained from each of the three layers as well as the weight of vascular material are included in Figure 3. Although all fractions roasted produced some typical peanut aroma and taste, the upper layer (aleurone grains and protein bodies) was by far the most potent in aroma and tasted very much like peanut butter.

Examination of these fractions under the microscope before and after heating revealed that the protein bodies and aleurone grains maintained their gross structural integrity throughout the heating process (180° C.) which was comparable to normal roasting temperatures (Figure 5). Heating caused clumping of particulates and lightening of the inclusion bodies, but the latter observation was not obvious from the photomicrographs in Figure 5. The starch grains (lower fraction of Figure 6*A*) ruptured during heating producing small donut-like holes in the center of the grain (Figure 6*B*) and an effervescence in the medium, apparently due to the release of water vapor. This effervescence began at about 110° C. That starch grains were the only particulates which ruptured was shown by examining particulates for typical birefringence.

These data established the protein-body aleurone grain fraction as the specific location of flavor precursors and implicated the aleurone grains more strongly than the protein bodies. However, this choice cannot be made until a good separation of these two bodies is effected, as judged by chemical analysis and by the presence of inclusion bodies. Chemically, the two differ in that aleurone grains have a much higher ash and phytic acid content (*2*).

Using this procedure to obtain a fraction rich in precursors followed by gel filtration for separation and purification of individual components promises to provide sufficient precursor material for the elucidation of structures.

Acknowledgment

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COTTONSEED FLOUR

Free and Total Gossypol, Epsilon-Amino Lysine, and Biological Evaluation of Cottonseed Meals and Flours in Central America

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Recently, Bressani *et al.* (*4–7*) have described the development of INCAP Vegetable Mixture 9, made of corn 28%, sorghum 28%, cottonseed flour 38%, torula yeast 3%, and dehydrated leaf meal 3%. This mixture, developed for the supplementary feeding of humans, had a high nutritive value (*15*). Its successful commercial production depends upon finding a cottonseed flour produced in Central America that is suitable for human consumption.

The specifications for human grade cottonseed flour, established in 1960 (*17*), required that it should contain not less than 50% protein, 3.6 grams of lysine per 16 grams of N, and not more than 0.055% of free gossypol and 1.00% of total gossypol. This paper reports on the differences in proximate composition, free ϵ-amino lysine, free and total gossypol, and nutritive value among cottonseed flours produced in the laboratory by screening commercial cottonseed meals.

Material and Methods

Five 400-pound samples of cottonseed meal, one from each of five mills located in Central America, were collected from the daily production and were brought to INCAP where they were stored at 4° C. until used. The samples were identified as follows: B, KH, AGSA, DS, and NC. Batches of 150 grams each from all the meals, each of which had different degrees of grinding, together with 10 small crystal balls, were placed on top of a series of screens (20, 40, 60, 80, 100 mesh) and shaken for 20 minutes in a laboratory shaker set at medium speed. After each run, the material which remained and that which had passed through the screens were weighed, and the percentage of each fraction was calculated. This process was continued until 1 kilogram of the lowest yielding fraction was obtained. Samples of the original meal and of each fraction were then analyzed for their proximate chemical composition by the A.O.A.C. official methods of analysis (*3*), and for their free and total gossypol, by the A.O.C.S. official methods (*2*). The free ϵ-NH_2 lysine content of each fraction was obtained by the method of Conkerton and Frampton (*9*).

For the biological trials, the original

Each of five samples of cottonseed oil meal produced in several countries of Central America was separated by simple screening into six granulometric fractions for the purpose of selecting those samples which could meet the composition specifications for human consumption. The original meal and the flour were analyzed for their chemical composition, free and total gossypol, and free ε-amino lysine. All samples, seven from each product, were then fed to young rats in diets providing 10% protein solely from cottonseed flour. By screening, it was demonstrated that the moisture, fat, nitrogen, and ash increased, while crude fiber decreased, as the particle size decreased. Both free and total gossypol and free ε-amino lysine, when expressed as percentage of the sample, were higher in the fractions with smaller particle size. PER was positively correlated with free ε-NH_2 lysine ($r = 0.705$; $\hat{Y} = -0.183 + 0.534X$), total gossypol and free ε-NH_2 lysine were negatively correlated ($r = -0.538$; $\hat{Y} = 5.78 - 2.17X$), and both free gossypol and total gossypol inversely affected the weight gain of the rats.

meal and all the fractions were tested in a basal diet calculated to provide 10% protein in the diet. The basal diet also contained 4% mineral mixture (*12*), 5% refined cottonseed oil, 1% cod liver oil, an amount of cottonseed flour equivalent to 10% protein, and sufficient cornstarch to adjust to 100%. All diets received 3 ml. of a complete vitamin mixture (*14*) per 100 grams of ration.

Six male weanling rats of the Wistar strain of the INCAP colony were used in each group. The various meals and their respective fractions were tested together, so that each experiment used a total of 42 rats. The animals were distributed by weight so that the average initial weight was the same for all groups, and they were placed in individual, all-wire screen cages with raised screen bottoms. Food and water were provided *ad libitum*, and the animal weights and food intakes were measured every 7 days for 28 days.

In one experiment, the fractions of each of the meals passing 40 mesh and above were compared with a high grade cottonseed flour in a biological trial similar to the one described above.

Table I. Proximate Composition of Five Central American Cottonseed Flours and Granulometric Fractions

Composition, %	Whole Meal	Particle Size, Mesh Coarser than: 20	40	60	80	100	Finer than 100
		COTTONSEED FLOUR B					
Moisture	11.0	9.7	9.2	10.7	10.4	10.5	10.3
Ether extract	3.4	3.0	3.3	3.8	4.0	4.1	4.0
Crude fiber	9.6	19.7	9.5	5.6	5.2	4.6	3.5
Nitrogen	6.19	4.71	6.48	7.42	7.38	7.50	8.02
Ash	6.6	5.6	6.8	8.8	7.5	7.7	7.6
		COTTONSEED FLOUR KH					
Moisture	8.2	10.3	9.2	11.4	11.5	11.5	11.3
Ether extract	6.0	4.0	4.8	5.5	5.6	6.1	6.0
Crude fiber	8.9	15.7	12.4	6.5	5.6	4.9	3.9
Nitrogen	6.74	5.45	6.37	7.42	7.77	7.61	7.58
Ash	6.6	5.6	6.2	6.8	6.9	7.0	7.2
		COTTONSEED FLOUR AGSA					
Moisture	6.7	6.6	6.0	6.5	6.8	7.2	7.6
Ether extract	5.0	4.0	4.0	4.5	4.9	5.7	6.5
Crude fiber	9.9	12.4	15.4	11.5	8.2	6.4	5.7
Nitrogen	7.40	7.01	6.62	7.19	7.81	8.10	7.79
Ash	7.2	6.6	6.4	8.2	7.7	7.9	8.1
		COTTONSEED FLOUR DS					
Moisture	9.4	9.1	8.3	9.7	10.0	9.8	9.7
Ether extract	6.7	5.9	6.2	7.1	8.0	7.5	7.9
Crude fiber	12.6	15.3	15.5	8.7	7.6	6.1	4.4
Nitrogen	6.28	5.86	5.98	7.31	7.45	7.20	7.40
Ash	6.5	6.2	6.0	7.0	7.2	7.2	7.3
		COTTONSEED FLOUR NC					
Moisture	7.7	7.1	6.9	8.4	8.5	8.8	8.7
Ether extract	7.5	6.3	6.5	7.9	8.2	8.4	8.6
Crude fiber	9.5	15.9	11.1	5.4	4.6	3.9	3.7
Nitrogen	6.88	5.55	6.67	7.86	7.40	7.70	7.70
Ash	6.4	5.8	6.5	7.1	7.2	7.4	8.2

Results

The per cent distribution of the fractions of each cottonseed meal is shown in Figure 1. The quantities which passed the various meshes were very variable; however, in general, lowest yields were in fractions with particles smaller than 60 and larger than 100 mesh.

The proximate chemical composition of the materials tested is shown in Table I. In general, the percentage of moisture, crude fat, nitrogen, and ash increased in all samples, while the crude fiber decreased as the size of the particle decreased.

The free and total gossypol and the free ε-NH_2 lysine content of the meals and their fractions are shown in Table II. In all cases, free gossypol increased as the particle size decreased, while total gossypol increased only in samples B and DS. In samples KH, AGSA, and NC, higher total gossypol values were observed in particles coarser than 60 to 100 than in particles finer than 100 mesh. There was a tendency for ε-amino lysine, expressed either as percentage of the sample or as grams per 16 grams of N, to increase to a peak value and then decrease as the particle size decreased. There were, however, some exceptions to this observation. Higher free ε-NH_2 lysine values were obtained in almost all cases in the fractions passing 60 and 80 mesh. Lower values were found in both fractions whether or not they passed 100 mesh. Nevertheless, all of the meals and their fractions separated according to particle size differed in free and total gossypol and free ε-NH_2 lysine content, and only one flour exceeded the ε-amino lysine content specifications (*17*). This flour was produced by prepress solvent extraction, while all the others were produced by the expeller process.

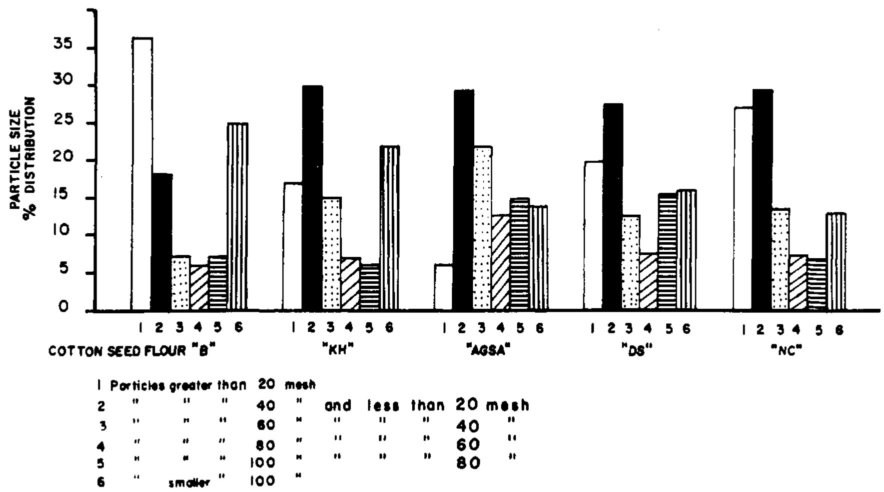

Figure 1. Particle size percentage distribution of Central American cottonseed flour

Table II. Total and Free Gossypol and Free ϵ-NH_2 Lysine Content of Central American Cottonseed Flours and Granulometric Fractions

Content	Whole Meal	Particle Size, Mesh Coarser than: 20	40	60	80	100	Finer than 100
			Cottonseed Flour B				
Free gossypol, %	0.064	0.055	0.060	0.064	0.063	0.060	0.071
Total gossypol, %	0.89	0.63	0.85	0.95	0.95	1.00	1.09
ϵ-NH_2 lysine, %	1.50	1.35	1.94	2.18	1.99	2.18	2.16
ϵ-NH_2 lysine, grams per 16 grams N	3.88	4.58	4.79	4.70	4.31	4.65	4.31
			Cottonseed Flour KH				
Free gossypol, %	0.044	0.035	0.034	0.047	0.048	0.050	0.054
Total gossypol, %	1.06	0.97	1.07	1.20	1.24	1.20	1.15
ϵ-NH_2 lysine, %	1.39	1.15	1.27	1.52	1.64	1.60	1.52
ϵ-NH_2 lysine, grams per 16 grams N	3.18	3.38	3.19	3.28	3.38	3.36	3.21
			Cottonseed Flour AGSA				
Free gossypol, %	0.044	0.036	0.031	0.039	0.043	0.053	0.055
Total gossypol, %	1.14	1.15	1.02	1.22	1.29	1.25	1.19
ϵ-NH_2 lysine, %	1.24	1.28	1.06	1.14	1.35	1.35	1.55
ϵ-NH_2 lysine, grams per 16 grams N	2.53	2.92	2.56	2.54	2.77	2.67	3.18
			Cottonseed Flour DS				
Free gossypol, %	0.044	0.038	0.036	0.048	0.052	0.056	0.059
Total gossypol, %	1.07	0.99	1.03	1.21	1.18	1.25	1.24
ϵ-NH_2 lysine, %	1.16	1.15	1.04	1.44	1.68	1.73	1.63
ϵ-NH_2 lysine, grams per 16 grams N	3.57	3.14	2.78	3.15	3.61	3.84	3.52
			Cottonseed Flour NC				
Free gossypol, %	0.049	0.040	0.039	0.056	0.057	0.073	0.080
Total gossypol, %	0.91	0.76	0.85	1.00	0.99	1.01	0.95
ϵ-NH_2 lysine, %	1.15	1.23	1.49	1.83	1.81	1.74	1.70
ϵ-NH_2 lysine, grams per 16 grams N	3.40	3.55	3.57	3.74	3.91	3.61	3.53

The results of the biological trials are shown in Table III. A tendency toward a slightly higher protein efficiency ratio (PER) is seen in the finer fractions in some cases, but this is not accompanied by greater weight gain. Of the five samples studied, only two gave satisfactory protein efficiency ratios, while the other three were of low protein value. The average weight gain of the rats in four out of five cases was lower with the particles passing 100 mesh, and these were the samples with higher free gossypol content and a high free ϵ-amino lysine value. The results of a comparison of all 40-mesh and above material fed at the 10% protein level in the diet indicated that the B and NC samples were superior to the other Central American samples as well as to the control cottonseed flour.

Discussion

The successful commercial production of vegetable protein mixture for the supplementary feeding of infants, young children, and adults requires that all or the majority of the ingredients be produced in the area or country where the mixture is to be used. The results presented here show that at least two cottonseed flours can be produced in Central America by a simple screening operation. The results further indicate that screening permits the production of cottonseed flour to meet most or all the specifications for the flour to be suitable for human feeding (*17*). The yields are, of course, variable, and depend upon the fineness of the meal before the screening; however, at least 35% of the original weight of the tested material was converted into satisfactory flour.

The screening operation gave some interesting results. The finer flours contained more protein, fat, and ash, due probably, to a large extent, to a reduction in the crude fiber content. Also, all the material that passed 80 mesh contained higher concentrations of free gossypol. Since, in many instances, these fractions represent relatively low percentages of the original weight of the cottonseed, they could be discarded. The material passing 40 and 60 mesh could be utilized for the preparation of the flour, which would probably contain less free gossypol while it had the other chemical components required to satisfy the specifications.

The PER figures of the biological tests were plotted against the free ϵ-NH_2 lysine values expressed as grams amino acid per 16 grams of N as shown in Figure 2. The regression equation for this relationship is $\hat{Y} = -0.183 + 0.534X$. A similar relationship has been reported by Mann *et al.* (*13*) and Frampton (*10*) in chicks, using a plot of available lysine and chick weight gain. This figure shows that the NC flour and the B flour are of high quality for nonruminant feeding. The first is prepared from good quality seed and by the expeller process, while the second is made by a prepress solvent extraction also using good quality seed. Figure 3*A* shows the relationship between total gossypol and free ϵ-amino lysine with a regression equation of $\hat{Y} = 5.78 - 2.17X$. Figure 3*B* shows plots of weight gain in relation to free gossypol content with individual correlation values. These figures indicate that for the samples made from the same cotton seed meal, the higher the gossypol the

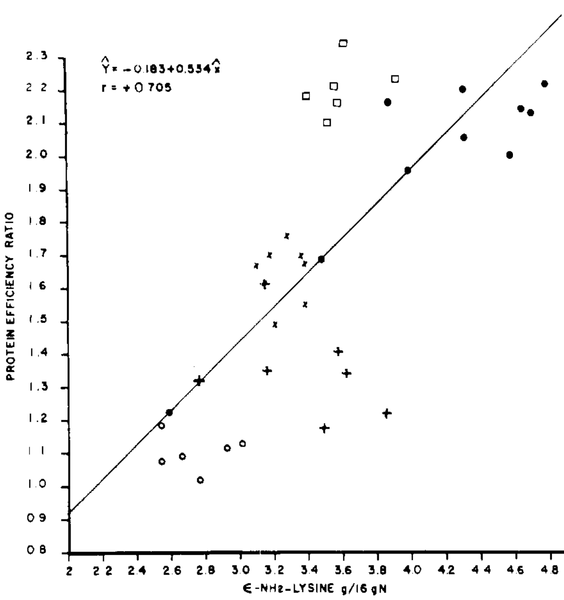

Figure 2. Relationship between ε-amino lysine (grams per 16 grams N) and protein efficiency ratio

□ = NC; ● = B; ○ = AGSA; × = KH; + = DS

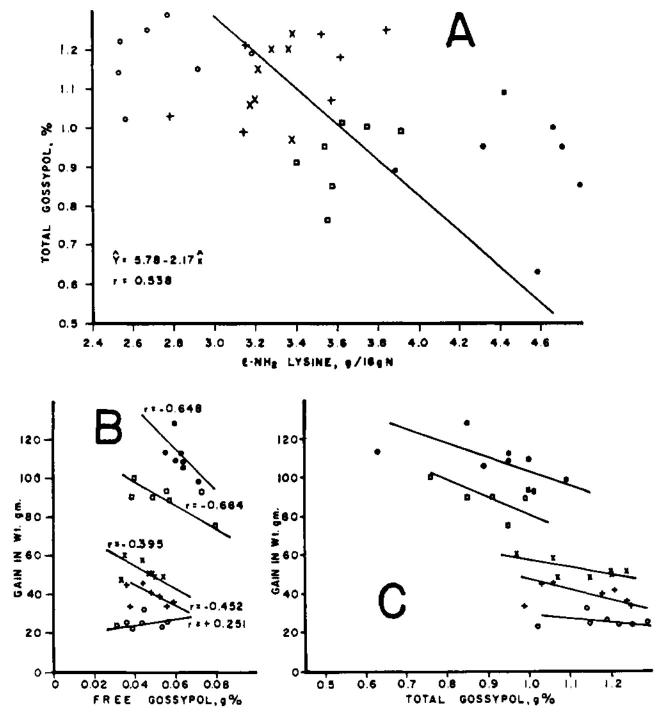

Figure 3. Relationship between gossypol and ε-amino lysine (A) and gossypol and weight gain (B,C)

□ = NC; ● = B; ○ = AGSA; × = KH; + = DS

lower the weight gain, suggesting independent effects of gossypol and of lysine, since available lysine was more or less constant when expressed in grams per 16 grams of N, and the gossypol content increased as the particle became smaller. Smith *et al.* (*16*), working with varieties of cottonseed having a low gossypol content, presented rat growth data and free gossypol content of several samples. When their values are plotted, a negative relationship between growth and gossypol is observed. Protein utilization, as expressed by PER, however, is not affected by free gossypol; it is dependent on free ε-amino lysine values. In some instances, the higher the free gossypol, the higher the PER. This is to be expected within a certain range, since it is well known that during cottonseed processing gossypol decreases available lysine, during the extraction of oil from cottonseed (*7*). Possibly PER is not affected by the free gossypol because the amount of free gossypol is directly related to the amount of fat in the cottonseed meals studied. Therefore, the extra gain in weight is probably due to the accumulation of body fat rather than to protein synthesis. This was also indicated by the lower consumption of the diets with higher free gossypol, which resulted in higher PER. In other words, there was a lower protein intake with an apparently better protein utilization, due really to a higher fat intake. The relationships between free gossypol and PER and free gossypol and weight gain are being studied further to distinguish the gossypol effect from the effect of decreased protein quality represented by free ε-amino lysine values. The results reported in this paper were obtained by using diets with 10% protein from cottonseed flour, and since gossypol effects decrease or disappear with higher protein levels (*8*, *11*), it would be of interest to learn whether the same relationship would be encountered at higher levels of protein intake.

Table III. Results of Rat Growth Experiments with Central American Cottonseed Flours and Their Granulometric Fractions

	Whole Meal	Particle Size, Mesh Coarser than: 20	40	60	80	100	Finer than 100
			Cottonseed Flour B				
Initial weight, grams	52	52	52	52	52	52	52
Weight gain, grams	106	113	128	108	112	109	98
Feed efficiency	4.3	4.2	3.8	4.1	4.0	4.2	4.5
PER	2.16	2.00	2.21	2.13	2.20	2.14	2.05
			Cottonseed Flour KH				
Initial weight, grams	42	42	42	42	42	42	42
Weight gains, grams	58	60	48	51	51	50	49
Feed efficiency	5.5	5.9	6.2	6.1	6.3	5.9	6.1
PER	1.70	1.55	1.67	1.76	1.68	1.70	1.49
			Cottonseed Flour AGSA				
Initial weight, grams	43	43	43	43	43	43	43
Weight gain, grams	32	25	23	23	25	24	26
Feed efficiency	8.3	8.9	11.1	9.0	9.7	9.1	8.3
PER	1.19	1.12	0.91	1.08	1.02	1.09	1.13
			Cottonseed Flour DS				
Initial weight, grams	48	48	48	48	48	48	48
Weight gain, grams	46	34	45	41	40	34	36
Feed efficiency	6.9	8.5	7.1	7.0	7.0	7.3	7.8
PER	1.41	1.62	1.32	1.35	1.34	1.22	1.18
			Cottonseed Flour NC				
Initial weight, grams	49	49	49	49	51	50	50
Weight gain, grams	90	100	90	93	89	93	75
Feed efficiency	4.7	4.5	4.8	4.5	4.5	4.6	4.7
PER	2.18	2.21	2.16	2.31	2.23	2.34	2.10

Total gossypol also seems to have some effect on growth, an effect which may be independent of free ε-amino lysine, as shown in Figure 3C. The DS sample, for example, has a higher total gossypol content than the NC sample, but it has similar free ε-amino lysine value; nevertheless, weight gain was much lower with the DS sample than with the NC sample. This observation, however, deserves further study.

Work along these lines is being continued to determine differences in effect between free and total gossypol, available lysine, and other nutritional defects in cottonseed meal and flour.

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FISH COMPOSITION

Proximate Composition of Silver Salmon

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Proximate composition of silver salmon was determined to obtain information about variations in composition from year to year, within the season, from fish to fish, and from different sections of individual fish. Average composition of edible flesh was: moisture, 72.7%; protein, 21.5%; oil, 5.73%; and ash, 1.2%. Silver salmon varied from very lean to oily—the amount of oil ranged from 1.6 to 12.5%. Fatty deposits were shown to exist in the belly flap, the dark meat along the side, and the dorsal layer along the back of the fish. These tissues had high concentrations of oil even when the entire edible flesh had a low amount of oil.

Silver salmon (*Oncorhynchus kisutch*) are found along the Pacific Coast from Monterey Bay, Calif., north to Kotzebue Sound in the Bering Straits, and in Asiatic waters as far south as Japan. The largest concentrations are found in Oregon, Washington, British Columbia, and southeastern Alaska. The salmon are marketed primarily as fresh, frozen, or canned fish. A fraction of the catch is smoked or kippered. Although silver salmon are important to sports and commercial fishermen, little has been reported on their composition or on variations in composition. Two extensive investigations have been made on the composition of canned silver salmon. Shostrom *et al.* (*3*) analyzed nine samples each from 11 localities in Alaska and Washington. The fish had been specially canned so that all samples were from the same section of the fish. Composition of fish from the different localities varied little except for the oil content, which ranged from 4.7 to 11.5%. Riddell (*2*) analyzed random samples of canned fish caught from May through September from one point off the British Columbia coast. Composition varied from month to month. The amounts of oil and protein were at a maximum in July. The present article

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Table I. Catch Information and Physical Data for Nine Lots, Each with 10 Troll-Caught Silver Salmon

Lot	Date Caught Month — Year	Weight, Pounds Av.	Weight, Pounds Range	Length, Inches Av.	Length, Inches Range
1	7 — 59	3.8	3.6–6.3	22.4	20.1–24.8
2	8 — 59	4.8	3.6–5.8	23.6	21.7–24.8
3	10 — 59	4.8	3.6–7.8	24.0	19.7–29.1
4	7 — 60	4.7	4.1–6.6	22.4	20.9–24.8
5	8 — 60	6.6	5.9–8.2	25.6	23.6–26.4
6	10 — 60	8.2	4.5–11.0	27.0	23.2–29.5
7	6 — 61	4.5	3.6–7.1	22.4	20.9–25.6
8	8 — 61	6.1	5.2–7.2	24.4	22.8–25.2
9	10 — 61	7.4	5.4–8.8	26.5	22.4–30.7