

VITAMIN B₁₂ SUPPLEMENTATION of the Diet of HEALTHY ADULTS

A STUDY OF WEIGHT CHANGES EMPLOYING A LATIN SQUARE DESIGN

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VITAMIN B₁₂ has been shown to produce a variety of effects in isolated tissues, bacteria, lower animals, and man. Among the more significant is the effect on growth and weight gain.

Vitamin B₁₂ supplementation has a dramatic effect upon the weight gain of the progeny of rats maintained during gestation and lactation on a vitamin B₁₂-deficient diet.¹ If the deficient diet of the mothers is supplemented with 5 µg. of vitamin B₁₂ per day, the enhanced weight gain in the treated young rats is only apparent in the last 30 days of a 90-day period of observation. This effect is thought to be related to vitamin B₁₂ storage. Weight gain is probably due to appetite stimulation,^{2,3} although this may not account for the entire effect.² It has been hypothesized that vitamin B₁₂ plays a role in the transformation of carbohydrate to fat.³ It has been shown also in mice⁴ and rats⁵ that the source of the calories may influence the amount of weight gain demonstrable during vitamin B₁₂ supplementation. In summary of the animal data, it can be said that weight gain has been demonstrated as a consequence of vitamin B₁₂ supplementation when there is (a) dietary deficiency including vitamin B₁₂ deficiency, and (b) *ad libitum* feeding. The degree of response may be influenced by the source of calories in the diet.

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Attempts to demonstrate a growth-promoting effect in humans have met with varying degrees of success.⁶⁻¹⁴ In general, the reports describing a positive effect upon weight gain or growth met the two requirements noted above for a positive effect in animals, whereas in many of the negative reports it is not certain that these criteria were met. It has been demonstrated in pregnant rats with the aid of vitamin B₁₂ containing radioactive cobalt (Co⁶⁰) that both orally and parenterally administered vitamin B₁₂ is transmitted to the placenta and to the fetus.³ Whether this obtains in humans is not known.

PURPOSE

We were interested in studying the effects of vitamin B₁₂ supplementation in middle-aged, healthy adults on an *ad libitum* diet where increased appetite and attendant weight gain might conceivably be a hazard to longevity rather than an index of good health, as it has been interpreted to be in early life.

In the past, when the function of a vitamin has been established, it has been incorporated with other known vitamins into multiple vitamin preparations. These are intended for prophylactic or therapeutic use during stressful situations where there is an increased vitamin requirement or a restriction in dietary intake. Multiple vitamin preparations are commonly used in association with low caloric diets in the therapy of obesity and are also

recommended in the management of pregnancy.

Although the foregoing information would not predict a response to vitamin B₁₂ in individuals without evidence of or reason for deficiency, the magnitude of the clinical problem of obesity,¹⁵ and the frequency of vitamin supplementation seemed to justify putting the problem to clinical test.

METHOD OF STUDY

At the Norristown State Hospital the circumstances were such that a sufficiently large number of individuals could be followed for long periods of time on an *ad libitum* diet which was similar for all patients. The subjects participating in this study, who were in normal physical health, had been originally institutionalized because of mental illness. At the time of this investigation all patients were housed in one building reserved for those under observation prior to release from the institution. Previously published information on the weight changes associated with vitamin B₁₂ administration indicated a need for reasonably large groups and the desirability of relatively long periods of study. These requirements seemed all the more important when dealing with groups in whom the likelihood of deficiency is slight. To further validate the observations for subsequent interpretation, the study groups were exposed to similar housing conditions and to the same diet. The vagaries of dietary intake were not felt to be any more varied in the patients selected for this study than would be anticipated for a similar-sized segment of the normal population. The influence on the study resulting from ability of the patient to select from the diet presented to him was minimized by the design of the study, which provided for the patient to be his own control.

Female patients from a single building who were fed in a common dining room were selected on the basis of the following criteria:

1. At least three months' residence in the building immediately prior to the initiation of the study and less than 5 lb. gain or loss within three months.

2. A likelihood of continued residence in the building for at least nine months.

3. No insulin shock or electroshock therapy during the period of study or during the three months prior to this.

4. No vitamin B₁₂ supplementation during the three-month period prior to this study.

If at any time after the start of the investigation an individual failed to fulfill these criteria, she was dropped from the study. At the beginning, there were 75 subjects, three groups of 25, and at the conclusion of the study 44 persons, groups of 16, 14, and 14, respectively. Since the design of this work allowed each individual to serve as her own control, the patients who completed the study constitute a significant number. Weights were recorded for each patient at monthly intervals to the nearest 1/4 lb. The patients were distributed within the three groups in such a manner as to assure comparable ages and initial weights. Fortunately, the necessity of eliminating individuals as the study progressed did not seriously modify the age or weight equality between groups (see Table II).

Each individual was allowed as much food as she desired at each regular mealtime. In order that variations among groups of patients and variations due to seasons of the year might be removed in the analysis of the data, a Latin square design¹⁶ was used (Table I).

TABLE I

Groups	Patients	Periods			
		Jan. 15- Apr. 15	Apr. 15- July 15	July 15- Oct. 15	Oct. 15- Jan. 15
I	1-25	—	A	B	C
II	26-50	—	B	C	A
III	51-75	—	C	A	B

Treatment Schedules

Each patient received each schedule of treatment for three months.

A = Placebo (different in appearance from either of the other two).

B = 100 µg. of vitamin B₁₂ once daily.*

C = Thirty cc. (30 µg.) of vitamin B₁₂ elixir given three times a day before meals.*

*"Redisol"® Tablets and "Redisol"® Elixir were generously supplied by Sharp & Dohme, Inc., Philadelphia, Penna.

Vitamin B₁₂ might influence appetite in a favorable direction either by relieving a deficiency state or by exerting a pharmacologic effect upon the utilization of ingested food. Accordingly, we compared a schedule of therapy in which vitamin B₁₂ was administered once daily with a schedule calling for the administration of vitamin B₁₂ before each meal.

RESULTS

The results were recorded as the increase or decrease in weight from the end of one treatment period to another ($Y_{x+1} - Y_x$). The final weight gains or losses are shown to the nearest $\frac{1}{4}$ lb. in Table II.

The mean results for groups, periods and treatments are shown in Table III.

TABLE II

Group I						
Patient	Age	Weight Y_0	Period (treatment)			
			I (A) Y_1 minus Y_0	II (B) Y_2 minus Y_1	III (C) Y_3 minus Y_2	
1	48	111	- 1.5	-4.25	2.5	
2	40	145	- 5.5	5.5	0.0	
3	50	136	0.75	3.25	4.5	
4	36	99	- 9.5	3.0	1.0	
5	53	147	- 3.0	3.5	-1.0	
6	54	122.75	0.5	-1.0	4.75	
7	55	122	- 2.5	-3.5	4.25	
8	52	109	- 3.0	2.0	3.5	
9	49	93	4.5	0.0	3.5	
10	42	102	- 2.5	1.5	1.5	
11	58	129	- 1.25	-3.75	4.5	
12	60	126	- 2.25	2.75	3.5	
13	45	105	- 2.75	2.25	1.25	
14	33	114	-10.5	4.0	-5.0	
Totals			-38.5	15.25	28.75	
Means	48.2	118.6	- 2.75	1.09	2.05	

Group II						
Patient	Age	Weight Y_0	Period (treatment)			
			I (B) Y_1 minus Y_0	II (C) Y_2 minus Y_1	III (A) Y_3 minus Y_2	
1	48	130	- 6.75	-4.0	1.0	
2	59	109.5	- 5.75	2.25	4.0	
3	50	133	0.5	2.5	4.75	
4	44	98	- 0.75	-1.25	-1.75	
5	53	138	- 5.5	2.0	2.75	
6	55	183	3.5	-3.5	10.0	
7	51	124	- 3.0	-3.75	1.5	
8	56	159.75	- 1.5	0.75	6.75	
9	49	126	- 3.75	2.75	-2.0	
10	42	123.75	0.5	1.25	1.25	
11	58	112	1.0	3.5	3.25	
12	62	98	- 2.0	0.0	3.5	
13	53	174	6.75	4.0	4.0	
14	44	134	4.0	-2.75	-3.25	
15	46	158	- 2.25	-3.75	3.5	
16	29	113	- 0.5	-1.5	2.5	
Totals			-15.5	-1.5	41.75	
Means	49.9	132.1	- 0.97	-0.09	2.61	

Group III					
Patient	Age	Weight Y ₀	Period (treatment)		
			I (C) Y ₁ minus Y ₀	II (A) Y ₂ minus Y ₁	III (B) Y ₃ minus Y ₂
1	40	103	-0.5	2.5	1.5
2	59	132.5	3.0	-0.25	-0.25
3	50	124.5	0.0	-1.75	-1.75
4	44	155.75	3.25	1.0	1.5
5	36	108.25	1.0	0.0	3.0
6	25	125	-4.75	1.75	-4.5
7	54	141.75	-3.75	-1.75	-1.5
8	41	147	5.5	10.5	2.0
9	51	112	-2.0	-2.0	4.0
10	47	187	2.75	-0.5	-0.5
11	42	136	-3.5	-3.25	-1.5
12	58	134	-0.5	1.0	3.0
13	63	159	0.5	2.5	4.25
14	32	93	-3.0	0.5	2.5
Totals			-2.0	10.25	11.75
Means	45.9	132.8	-0.14	0.73	0.84

TABLE III

Analysis	of	Weight Gain Lb.
Treatment		Mean results
A		0.31
B		0.26
C		0.57
Period		Mean results
I		-1.27
II		0.55
III		1.87
Groups		Mean results
I		0.13
II		0.52
III		0.48

The mean weight gains were so nearly identical for the three treatments that no statistical analysis seemed necessary. The three groups of patients likewise showed comparable weight gains. The variation among periods probably reflects seasonal effects, the largest weight gains occurring in Period III, which included fall and early winter.

DISCUSSION

These results illustrate the advantage of the particular experimental design employed. For example, the results for Group I show the following weight changes: (A) placebo, -2.75

lb.; (B) 100 μ g. vitamin B₁₂ once daily; +1.09 lb.; and (C) 30 μ g. vitamin B₁₂ three times a day, +2.05 lb. One would obtain a definite impression that vitamin B₁₂ had a favorable effect. However, the results with Groups II and III would indicate that the apparent beneficial effect of vitamin B₁₂ may be a reflection of an influence of seasons.

Likewise, the results for the three groups at the end of the first period are: (A) -2.75 lb.; (B) -0.97 lb.; and (C) -0.14 lb. Once again, there is a suggestion that vitamin B₁₂ may be showing a favorable effect. However, the apparent favorable result does not persist in either of the last two periods. As a matter of fact, the placebo (A) showed the largest mean weight gain, +2.61 lb., in period III.

By using a Latin square design we were able to average out the effect of seasons and of groups of individuals, thereby obtaining a less biased comparison of the three treatments.

Conclusions cannot be drawn regarding the relative merits of the two schedules of vitamin B₁₂ therapy employed, because neither schedule produced any weight gain in the individuals selected for study. Indeed, the question of appetite stimulation by vitamin B₁₂ in adult human subjects, except as it has been dealt with in this study, remains unanswered. It remains for someone to employ larger oral

doses of vitamin B₁₂ (1000 µg. or more) and to administer vitamin B₁₂ parenterally before it can be stated that this factor does not stimulate appetite in the human adult.

Despite the negative results of the study here reported, this vitamin does exert profound metabolic effects that are beyond question.

(a) Vitamin B₁₂ is necessary for the maintenance of a normal hematologic state by reason of its participation in nucleic acid synthesis. A part, if not the whole, of its activity in this regard is attributed to facilitation of the conversion of the pyrimidine base thymine to the nucleoside thymidine.¹⁷ (b) The influence of vitamin B₁₂ upon the integrity of the nervous system is attested to by the relief of combined system disease seen in pernicious anemia, whereas folic acid, which corrects the hematologic aspects of pernicious anemia, exerts no beneficial effects upon neurologic manifestations of this disease.¹⁸ (c) A lipotropic effect has been clearly demonstrated in animals and may be related to the participation of vitamin B₁₂ in certain transmethylation reactions.¹⁹ In view of these demonstrated activities of vitamin B₁₂, its inclusion in polyvitamin preparations seems reasonable. On the basis of the study here reported, it seems unlikely that quantities of vitamin B₁₂ up to 100 µg. per day will have any significant effect upon the appetite of adult humans, but further studies on the effects of vitamin B₁₂ in adult nutrition seem fully justified.

SUMMARY

Two schedules of oral vitamin B₁₂ supplementation, namely, 100 µg. daily for 3 months and 30 µg. three times a day for 3 months were evaluated in conjunction with a placebo given daily for 3 months. There was no significant weight gain attributable to vitamin B₁₂ administration in this group of 44 healthy adult females given an *ad libitum* diet for the 12-month period encompassed by the control and treatment phases of the study.

There was no significant difference in the weight gain during the period when the patients received a daily dose of 100 µg. of

vitamin B₁₂ as compared with the period when they received 30 µg. three times per day.

The value of the Latin square design is emphasized as a valuable aid in the interpretation of a long-term study such as this, where a number of uncontrollable variables are involved.

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RESUMEN

Adición de vitamina B₁₂ a la dieta de adultos sanos: un estudio de cambios de peso empleando un esquema cuadrado latino

Dos regímenes de adición de vitamina B₁₂ por vía oral, es decir, 100 microgramos por día durante 3 meses, y 30 μ gms. 3 veces por día durante 3 meses, se valoraron en conjunto con un placebo suministrado todos los días durante 3 meses. No se observó ningún aumento importante de peso que se podía atribuir a la administración de vitamina B₁₂ en este grupo de 44 mujeres adultas sanas recibiendo una dieta *ad libitum* durante el período de 12 meses abarcado por las fases control y experimental de este estudio.

No hubo ninguna diferencia importante en el aumento de peso durante el período que los pacientes recibían una dosis diaria de 100 μ gms. de la vitamina, en comparación con el período en que recibían 30 μ gms. 3 veces por día.

Se insiste en el valor del esquema cuadrado latino como un auxilio de gran importancia en la interpretación de una investigación prolongada como ésta, en donde se encuentran factores tan variados e incontrolables.

