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Hypocholesterolemic effect of vegetable protein in a hypocaloric diet

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Summary

Eleven obese volunteers took part in a 12-week study during 8 weeks of which 2 meals of their control 1000 kcal diets were replaced by a soya based liquid formula (1 month) or a milk based liquid formula (1 month). The mean weight loss per month was 2.5 kg ($P < 0.05$). On the soya formula total and LDL cholesterol levels were reduced significantly over the month by $10.0 \pm 2.7\%$ ($P < 0.01$) and $17.5 \pm 5.6\%$ ($P < 0.02$), respectively. Neither the milk based formula or the control low calorie diet lowered serum cholesterol significantly over the diet period. No change was seen in serum triglycerides on any of the 3 diets. No difference was seen between treatments in 24-h urinary C-peptide excretion. The results indicated that use of a vegetable protein supplement in a weight loss program which induced moderate weight loss was associated with a reduction in blood lipids, whereas moderate weight loss on a control low calorie diet or milk based formula was not.

Key words: Vegetable protein; Hypocaloric diet; Weight loss; Blood lipids

Introduction

There is evidence that substantial weight gain results in a rise in serum lipids [1,2] while major weight loss is associated with reduced serum lipids [3,4]. However, the anticipated lipid changes are

not always seen where the change in weight is moderate [5,6] and there are even reports where major weight loss was without effect [7]. Substitution of vegetable for animal protein has been shown to result in lower blood lipids [8-11]. Whether this occurs at relatively low levels of vegetable protein intake is not known.

The aim of the present study was therefore to look at the effect of moderate vegetable protein supplementation on blood lipids against a background of modest weight loss in obese volunteers.

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Methods

Eleven obese women (38 ± 4 years, $155 \pm 7\%$ of desirable weight [12], range 120–192%) volunteered for the study (Table 1). All volunteers were otherwise in good health, on no medications and specifically they had no history or evidence of cardiac, renal or thyroid disease or diabetes. The total period of observation and planned adherence to a reduced calorie intake was 3 months. Each month represented a separate study period. These included 1-month periods on a conventional reducing diet (1000 kcal) (low calorie control), or on the same diet except that a soya based supplement (soy) was taken in place of 2 regular low calorie meals daily. The soy supplement (Slim Plan Drink Mix, Shaklee Canada Inc., Burlington, Ont. L7L 5P2) was supplied as a powder (25 g) in a sachet to be mixed with 250 ml 2% butterfat milk. Each sachet provided 8.7 g soy protein, 9.1 g carbohydrate, 1 g fiber and 3.0 g fat with a P/S ratio of 2.4. The ingredients are shown in Table 2. The order of the first 2 periods was randomized. During the last month a milk based supplement (milk) was taken in place of the soy supplement. The

milk supplement was also supplied as a powder (25 g) in a sachet to be mixed with 250 ml 2% butterfat milk (Shaklee Corporation, Hayward, CA 94540). Each 25-g sachet provided 8.8 g milk protein, 7.7 g carbohydrate, 1 g fiber (largely β -glucan), and 3.0 g fat (soya) with a P/S ratio of 2.4. The ingredients are shown in Table 2. The full fat soy flour used in the soy formula was reconstituted from defatted soya flour and partially hydrogenated soya oil. The same oil was added in the same amount to provide the fat source in the milk formula. The available carbohydrate in the milk formula was largely endogenous lactose and added maltodextrins and starch compared to endogenous sucrose and added fructose in the soy formula. The vitamin and mineral mix differed in that the soy supplement had been developed for the Canadian market and the milk supplement vitamin and mineral mix for the Japanese market. Consequently Ca^{2+} and Mg^{2+} were higher in the soy formula and trace elements and vitamins (A, D, E, C, pantothenic acid and niacin) were higher in the milk formula. Furthermore DL-methionine had been added to the milk supplement (Table 2). Throughout the entire period patients were main-

TABLE 1

PATIENT DETAILS WHERE GROUP I TOOK THE SOY DIET FIRST AND GROUP II TOOK THE CONVENTIONAL DIET FIRST

| | Age (yrs) | Height (cm) | Initial weight (kg) | % ideal weight | Body mass index (kg/m^2) | Initial skinfold thickness | |
|----------|--------------|----------------|---------------------------|-------------------|--|----------------------------|---------------------|
| | | | | | | Triceps (mm) | Subscapular (mm) |
| Group I | | | | | | | |
| 1 | 24 | 170 | 71.3 | 120 | 24.7 | 31.1 | 20.8 |
| 2 | 56 | 165 | 74.0 | 132 | 37.3 | 58.6 | 41.4 |
| 3 | 27 | 172 | 110.3 | 181 | 37.3 | 50.2 | 51.4 |
| 4 | 23 | 168 | 97.9 | 168 | 34.7 | 41.4 | 45.0 |
| 5 | 25 | 167 | 87.5 | 152 | 31.4 | 43.8 | 47.5 |
| 6 | 34 | 173 | 117.8 | 192 | 39.4 | 50.4 | 60.0 |
| Mean | 31.5 | 169 | 93.1 | 158 | 32.5 | 46.5 | 44.4 |
| SEM | 5.2 | 1.2 | 7.7 | 11 | 2.3 | 4.0 | 5.4 |
| Group II | | | | | | | |
| 7 | 54 | 163 | 88.1 | 161 | 33.2 | 47.8 | 49.2 |
| 8 | 49 | 174 | 107.1 | 172 | 35.5 | 47.3 | 48.4 |
| 9 | 49 | 161 | 80.0 | 150 | 30.9 | 54.8 | 59.2 |
| 10 | 56 | 174 | 89.5 | 143 | 29.6 | 49.0 | 49.0 |
| 11 | 26 | 176 | 86.3 | 136 | 27.9 | 54.2 | 41.6 |
| Mean | 45.6 | 170 | 90.2 | 152 | 31.4 | 50.6 | 49.6 |
| SEM | 5.4 | 3.1 | 4.5 | 6.4 | 1.3 | 1.6 | 3.6 |

TABLE 2
INGREDIENTS OF SOY AND MILK BASED FORMULAE

| Ingredients | Soy formula (g/100 g) | Milk formula (g/100 g) |
|-----------------------------------|--------------------------|---------------------------|
| Soy protein isolate ^a | 31.53 | - |
| Full fat soy flour ^b | 23.40 | - |
| Milk protein isolate ^c | - | 34.18 |
| Non-fat dry milk | - | 13.00 |
| Fructose | 23.00 | 6.05 |
| Oat flour | 9.80 | 9.80 |
| Maltodextrin | - | 8.02 |
| Partially hydrogenated soy oil | - | 7.75 |
| DL-Methionine | - | 4.14 |
| Lecithin | 2.50 | 2.50 |
| Yeast | 1.90 | 6.65 |
| Rose hip powder | 1.75 | 1.75 |
| Carrageenan | 1.55 | 1.55 |
| Flavors | 1.02 | 1.02 |
| Sodium chloride | 0.68 | - |
| Vegetable fat | 0.50 | 0.50 |
| Calcium carbonate | 0.58 | - |
| Minerals ^d | 1.64 | 1.46 |
| Vitamins ^e | 0.15 | 1.63 |

^a Soy protein isolate (SUPRO 610K, Shaklee Corporation, CA) 86.0% protein, 0.1% fat.

^b Full fat soy flour (NUTRISOY 101, Shaklee Corporation, CA) 33.5% protein, 34.5% fat, 26.3% carbohydrate.

^c Milk protein isolate (TMP 1310, New Zealand Milk Products, Petaluma, CA 94952) 89.7% protein, 1.1% fat, 0.2% carbohydrate.

^d The increase in minerals on soy was due to the higher Mg²⁺ levels.

^e The increased weight of vitamins in the milk formula reflected increased amounts of vitamin C, 0.577 g; niacin, 0.109 g; pantothenic acid, 0.09 g; and vitamin E, 0.242 g.

tained on a 1000 kcal/day diet. The supplements, with a caloric density of approx. 400 kcals/100 g, therefore represented 20% of the daily caloric intake. Table 3 shows a typical days menu from each of the 3 treatments (soy, control and milk) from a volunteer whose lipid results approximated closely the group mean. The nutrient intakes for each treatment were calculated from food tables [13] and mean results for the group are shown in Table 4. The total vegetable protein intakes for the three periods were: soy, 28 ± 1 g/day, conventional 16 ± 2 g/day, milk 12 ± 1 g/day. Similarly the animal protein intakes were 44 ± 2, and 39 ± 3 and 57 ± 5 g/day, respectively. Total intakes of fat and carbohydrate were similar for the 3 treat-

ments although the cholesterol intake was higher during the low calorie control month (Table 4), and the proportion of vegetable protein was higher on the soya diet.

All patients were seen at weekly intervals throughout the study. Weight was measured at each visit and subscapular and mid triceps skinfold thickness was measured using John Bull calipers (British Indicators Ltd., England). In addition, 5-7 day food intakes, recorded by the patients during the previous week, were presented to the dietitian for discussion with the volunteer. When necessary, dietary modifications were made in the light of body weight measurements. These records were then used for estimation of nutrient intakes over the study periods (Table 4). A fasting blood sample was taken every 2 weeks. In addition, 24-h urine samples were obtained once a month.

Blood samples were analyzed for total and HDL-cholesterol [14,15] and triglyceride [16]. LDL-cholesterol was derived from the other lipid values [17]. Urine was analyzed for C-peptide [18] and creatinine [19], the full results of which will be reported in detail elsewhere (Wolever, T.M.S., unpublished).

Results were expressed as means ± SEM and significance of differences across treatments was assessed by analysis of variance and Student's *t*-test for paired data. Correlation coefficients were determined by regression analysis. No differences were observed between the lipid values for week 2 and 4 on any treatment. Means of these values are therefore given in Tables 5 and 6 for each treatment period.

Finally we determined whether increased P/S ratio, lower dietary cholesterol intakes and differences in weight reduction could account for the fall in cholesterol seen on the soy period. These variables were substituted into the equation used to predict serum cholesterol changes which had been derived from Lipid Research Clinics Coronary Primary Prevention trial data [32]. Here the predicted change (Δ) in serum cholesterol in mg/dl (ΔC) was:

$$\Delta C = 102.1 \Delta Q + 0.73 \Delta SF - 0.43 \Delta PF \\ + 0.014 \Delta DC - 17.8 \text{ mg/dl}$$

TABLE 3

TYPICAL DIET RECORD FOR ONE DAY ON EACH OF THE 3 TREATMENT PERIODS FOR A VOLUNTEER WHOSE LIPID RESPONSE CLOSELY REFLECTED THE MEAN RESPONSE OF HER PEERS

| | Soy | Low calorie control | Milk |
|-----------|--|---|---|
| Breakfast | 1 soy meal replacement 1 cup 2% milk 1 banana | 1 slice wholewheat bread 2 tsp peanut butter 1/2 cup 2% milk | 1 milk meal replacement 1 cup 2% milk |
| Lunch | 1 soy meal replacement 1 cup 2% milk 1/2 banana | 4 melba toast 2 tbsp tuna green salad 1 med. apple | 1 milk meal replacement 1 cup 2% milk |
| Snack | 1 bran muffin | 1 bran muffin | |
| Dinner | 3 oz hamburger 1/2 cup boiled rice 1/2 cup carrots 1 cup broccoli 1 tsp butter | 3 oz hamburger 1/2 cup boiled rice 1/2 zucchini 1 cup green beans 1 cup 2% milk | 3 oz hamburger 1/2 cup cooked spaghetti 1 cup cabbage 1 tsp butter 1/2 cup tomatoes lettuce and diet salad dressing |
| Snack | 1 diet drink | 1 cup orange juice | 1 grapefruit |

TABLE 4

NUTRIENT COMPOSITION OF THE 4 DIETS

| Treatment | Protein | | Fat | | Cholesterol (mg) |
|-----------------------|-----------------------|--------------------------|---------------------|-------------|-------------------------|
| | Total (g) | % Vegetable | Total (g) | P/S ratio | |
| Baseline ^a | 78 ± 6 ^a | 27 ± 3 ^a | 68 ± 8 ^a | 0.33 ± 0.06 | 383 ± 43 ^a |
| Control | 55 ± 2 ^b | 29 ± 3 ^a | 32 ± 3 ^b | 0.25 ± 0.03 | 408 ± 42 ^a |
| Milk | 71 ± 2 ^a | 17 ± 2 ^b | 36 ± 2 ^b | 0.30 ± 0.02 | 195 ± 23 ^b |
| Soya | 72 ± 2 ^a | 39 ± 1 ^c | 37 ± 2 ^b | 0.32 ± 0.03 | 214 ± 35 ^b |
| | Carbohydrate (g) | | | Calories | |
| | Available | Fiber | | | |
| Baseline ^a | 176 ± 23 ^a | 9.9 ± 1.4 ^{ab} | | | 1656 ± 175 ^a |
| Control | 120 ± 9 ^b | 13.7 ± 1.4 ^b | | | 990 ± 56 ^b |
| Milk | 111 ± 8 ^b | 12.4 ± 1.4 ^{ab} | | | 1058 ± 40 ^b |
| Soya | 117 ± 8 ^b | 9.9 ± 1.4 ^a | | | 1090 ± 45 ^b |

Means of each nutrient with different letter supercripts are significantly different ($P < 0.05$).

^a Baseline represents the diet before the start of the study.

TABLE 5

MEAN ± SEM BODY WEIGHT (kg), SERUM LIPIDS (mmol/l) (MEAN OF WEEKS 2 AND 4) AND 24 h URINARY CREATININE (mmol) AND C-PEPTIDE (pmol/mmol CREATININE) EXCRETION AT THE END OF EACH MONTH OF THE 3 MONTHS WITH THE RESULTS OF ALL TREATMENTS POOLED

| | Baseline | 1st month | 2nd month | 3rd month |
|-------------------|--------------------------|---------------------------|---------------------------|---------------------------|
| Body weight | 92.8 ± 4.5 ^A | 87.7 ± 4.2 ^B | 86.4 ± 4.4 ^B | 84.4 ± 4.2 ^C |
| Total cholesterol | 5.11 ± 0.43 ^a | 4.75 ± 0.45 ^{ab} | 4.56 ± 0.33 ^b | 4.75 ± 0.37 ^{ab} |
| LDL-cholesterol | 3.25 ± 0.34 ^a | 2.98 ± 0.41 ^{ab} | 2.66 ± 0.29 ^b | 2.93 ± 0.33 ^{ab} |
| HDL-cholesterol | 1.33 ± 0.07 | 1.28 ± 0.07 | 1.36 ± 0.08 | 1.26 ± 0.05 |
| Triglyceride | 1.15 ± 0.18 | 1.08 ± 0.10 | 1.21 ± 0.17 | 1.24 ± 0.16 |
| Urine C-peptide | 2.42 ± 0.26 ^a | 1.56 ± 0.17 ^b | 2.02 ± 0.30 ^{ab} | 1.60 ± 0.18 ^b |
| Urine creatinine | 10.7 ± 1.06 | 10.9 ± 0.87 | 9.1 ± 0.80 | 10.6 ± 1.23 |

Values on the same line not sharing the same letter are significantly different: small letters, $P < 0.05$; capital letters, $P < 0.01$.

TABLE 6
WEIGHT LOSS ON INDIVIDUAL DIETS

Group I took the soy diet first and Group II took the low caloric control diet first.

| | Weight loss (kg) per month | | |
|----------|----------------------------|------|------|
| | 1st | 2nd | 3rd |
| Group I | | | |
| 1 | 3.1 | 1.1 | 0.4 |
| 2 | 1.1 | 1.4 | 1.6 |
| 3 | 6.1 | 4.6 | 5.8 |
| 4 | 7.1 | 1.1 | 0.1 |
| 5 | 5.9 | -1.6 | 0.9 |
| 6 | 6.4 | -0.1 | 2.6 |
| Mean | 5.0 | 1.1 | 1.9 |
| SEM | 1.0 | 0.8 | 0.9 |
| Group II | | | |
| 7 | 4.9 | 2.5 | 1.0 |
| 8 | 1.9 | -2.0 | 3.5 |
| 9 | 3.4 | 2.8 | 1.8 |
| 10 | 0.7 | 3.6 | -0.5 |
| 11 | 4.4 | 1.6 | 5.1 |
| Mean | 3.1 | 1.7 | 2.3 |
| SEM | 0.8 | 1.0 | 1.0 |

where

$$\Delta Q = \Delta QI \left[1 - 1.148 \Delta QI - 8.72 (\Delta QI)^2 + 4.7 (\Delta QI)^3 + 15.4 (\Delta QI)^4 \right]$$

QI is the Quetelet index in g/cm²; SF is 24-h intake of saturated fat as a percentage of total calories; PF is the corresponding figure for polyunsaturated fat; and DC is the dietary cholesterol in mg per day.

TABLE 7

MEAN \pm SEM BODY WEIGHT LOSS (kg), SERUM LIPIDS (mmol/l) (MEAN OF WEEKS 2 AND 4) AND 24-h URINARY CREATININE (mmol) AND C-PEPTIDE (pmol/mmol CREATININE) EXCRETION AT THE END OF EACH MONTH OF THE 3 MONTHS FOR THE INDIVIDUAL TREATMENTS (SOY, CONVENTIONAL AND MILK)

| | Baseline | Soy | Conventional | Milk |
|-------------------|------------------------------|-------------------------------|-------------------------------|-------------------------------|
| Body weight loss | - | 3.5 \pm 0.8 | 2.0 \pm 0.6 | 2.0 \pm 0.6 |
| Total cholesterol | 5.11 \pm 0.43 ^a | 4.53 \pm 0.38 ^b | 4.79 \pm 0.44 ^{ab} | 4.75 \pm 0.37 ^{ab} |
| LDL-cholesterol | 3.25 \pm 0.34 ^a | 2.72 \pm 0.34 ^b | 2.92 \pm 0.37 ^{ab} | 2.93 \pm 0.33 ^{ab} |
| HDL-cholesterol | 1.33 \pm 0.07 | 1.32 \pm 0.06 | 1.32 \pm 0.09 | 1.26 \pm 0.05 |
| Triglyceride | 1.15 \pm 0.18 | 1.07 \pm 0.11 | 1.22 \pm 0.15 | 1.24 \pm 0.16 |
| Urine C-peptide | 2.42 \pm 0.26 ^a | 1.79 \pm 0.23 ^{ab} | 1.79 \pm 0.25 ^{ab} | 1.60 \pm 0.18 ^b |
| Urine creatinine | 10.7 \pm 1.06 | 10.7 \pm 0.91 | 9.2 \pm 0.79 | 10.6 \pm 1.23 |

Values on the same line not sharing the same letter are significantly different, $P < 0.05$.

Results

Table 5 shows the mean body weight and serum lipids in the total cohort of volunteers over the 3-month period independent of treatment. Weight fell consistently over the entire period with significant drops over the first and last months. On the other hand, the total cholesterol and LDL-cholesterol levels did not change significantly across the 3-month period despite transiently significant lower levels at the end of the second month.

When the results were assessed according to treatment, weight losses of 3.5 ± 0.83 , 2.0 ± 0.63 and 2.0 ± 0.61 kg were seen after 1 month on soya, control and milk diets, respectively. The weight loss on soya, although greatest, was not significantly different from the other treatments (Tables 6 and 7).

Only during the soya period did the absolute levels of total and LDL-cholesterol (mean of 2-week and 4-week) fall significantly below the common baseline values obtained at the start of the 12-week study (Table 7). When groups I and II were assessed independently the soy month showed significant reductions in total cholesterol below baseline values independent of whether soy was taken in the first month (group I) or second month (group II) of the study (Table 8). Pooling the data from both groups demonstrated falls by the end of the soy month of $10.0 \pm 2.7\%$ ($P < 0.01$) for total cholesterol and $17.5 \pm 5.6\%$ ($P < 0.02$) for LDL-cholesterol when compared to baseline. Significant reductions in total and LDL-cholesterol

TABLE 8

MEAN TOTAL CHOLESTEROL LEVELS IN GROUPS I AND II FOR BASELINE AND MONTHS 1, 2 AND 3 DEMONSTRATING THE ORDER OF TREATMENTS IN EACH GROUP

Percentage differences from baseline values (% Δ) are also given for each treatment period.

| | Baseline (mmol/l) | Month 1 soya | | Month 2 control | | Month 3 milk | |
|---------------------|----------------------|-----------------|------------------------------------|-----------------|-----------------------------------|-----------------|----------------------|
| | | mmol/l | % Δ | mmol/l | % Δ | mmol/l | % Δ |
| Group I (n = 6) | 4.74 \pm 0.73 | 4.11 \pm 0.61 | -12.2 \pm 4.0 <i>P</i> < 0.05 | 4.18 \pm 0.51 | -9.4 \pm 4.2 NS | 4.45 \pm 0.63 | -3.6 \pm 6.3 NS |
| | Baseline (mmol/l) | Month 1 control | | Month 2 soya | | Month 3 milk | |
| | | mmol/l | % Δ | mmol/l | % Δ | mmol/l | % Δ |
| Group II (n = 5) | 5.54 \pm 0.34 | 5.53 \pm 0.54 | -0.9 \pm 5.9 NS | 5.04 \pm 0.35 | -9.4 \pm 1.6 <i>P</i> < 0.01 | 5.11 \pm 0.28 | -7.4 \pm 3.4 NS |

were not seen at the end of either the low calorie control or milk periods. Furthermore a similar picture emerged when the changes were examined by month rather than by comparison with the common baseline value. Again, after pooling data from groups I and II, the starting LDL-cholesterol value for volunteers on soy was 3.4 \pm 0.4 mmol/l and the 2-week and 4-week values were significantly reduced at 2.7 \pm 0.3 mmol/l (*P* < 0.001) and 2.8 \pm 0.4 mmol/l (*P* < 0.05) respectively. The 3 corresponding values on the low calorie control

were: 3.0 \pm 0.4, 2.8 \pm 0.4, and 3.1 \pm 0.4 mmol/l, and on milk the values were 2.7 \pm 0.3, 2.9 \pm 0.3, and 2.9 \pm 0.4 mmol/l, and there were no significant differences. The percentage changes with time are shown for each treatment in Fig. 1.

For serum triglyceride levels, no significant differences were seen from baseline values after any of the treatments (Table 7).

Urinary C-peptide excretion fell significantly from baseline over the first month of the study, and tended to remain at the new low level for the subsequent 2 months (Table 6). C-peptide excretion was not significantly different for any of the 3 dietary periods (Table 7). Changes in urinary C-peptide excretion were significantly related to changes in weight (Δ Wt) on the soya diet when

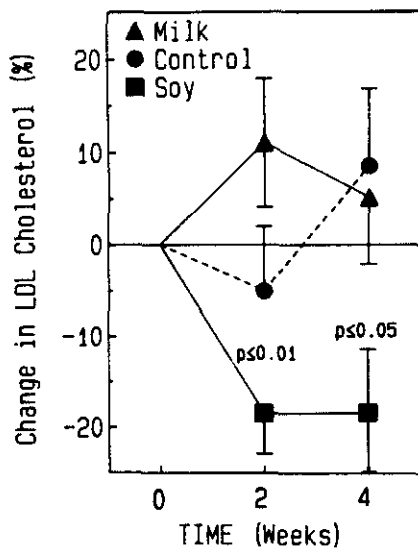


Fig. 1. Percentage changes from initial values at the start of each month for LDL cholesterol across the three treatments: soy (■—■); low calorie control (●- - -●); and milk (▲—▲).

TABLE 9

CHANGE IN SERUM CHOLESTEROL ON THE 3 DIETARY PERIODS AS OBSERVED AND AS PREDICTED^a ON THE BASIS OF WEIGHT CHANGE AND DIETARY CHANGE IN CHOLESTEROL, SATURATED AND POLY-UNSATURATED FAT INTAKES

| | Change in serum total cholesterol (mg/100 ml) | | |
|------------------------------------|--|-----------------|-----------------|
| | Soy | Control | Milk |
| Observed | -22.8 \pm 4.8 | -12.1 \pm 8.5 | -14.0 \pm 6.6 |
| Predicted | -8.1 \pm 1.4 | -13.1 \pm 1.8 | -12.5 \pm 2.8 |
| Significance of differ- ence | <i>P</i> < 0.05 | NS | NS |

^a Based on LRC equation [32].

expressed as percentage changes from baseline ($\% \Delta$) ($r = 0.851$, $n = 11$, $P < 0.001$) and also when all monthly changes were combined ($\% \Delta$ C-peptide = $8.48\% \Delta$ Wt - 11.1 ; $r = 0.533$; $n = 33$; $P < 0.01$).

Changes in lipids related to changes in body weight, cholesterol and fat intakes

The LRC equation, which takes into consideration changes from baseline in weight, dietary cholesterol, saturated and polyunsaturated fat, accurately predicted the observed changes in serum total cholesterol seen on the low calorie control and milk formula diets (Table 9). Using these data, however, it significantly underestimated the fall in serum total cholesterol seen on the soy diet by 14.7 ± 5.7 mg/dl ($P < 0.05$).

There were no significant relationships between weight change and changes in individual serum lipids after any of the treatments. However, when all monthly changes were combined, changes in weight were significantly related to changes in serum triglyceride (TG) such that a weight loss of 5 kg was associated with a reduction of triglycerides of 0.34 mmol/l ($\% \Delta$ TG = $0.068\% \Delta$ Wt - 0.199 ; $r = 0.427$; $n = 33$; $P < 0.05$).

Discussion

The present study confirms the effect of substitution of vegetable for animal protein in reducing serum lipid levels. However, these comparisons were undertaken against a background of consistent negative caloric balance unlike those of previously reported studies. The absolute amounts of protein consumed on both diets was therefore smaller than in previous reports. In addition it may be possible for the co-ingestion of animal protein to mask the effect of small amounts of vegetable protein on blood lipids. Furthermore, since the hypolipidemic effect of vegetable protein is usually most apparent in hypercholesterolemic subjects [8] there was a further reason why normocholesterolemic individuals may display no lipid lowering action with weight loss. The selection of predominantly normolipidemic subjects here, together with the higher proportion of animal protein, may also explain why only a modest effect was seen on serum cholesterol with the use

of the soy protein supplement. Previous workers have suggested a number of reasons why vegetable proteins may reduce serum lipids including an alteration in the ratio of dietary arginine to lysine [10] or a reduction in methionine intake [21]. It is also possible that reduced insulin secretion rates may have reduced the stimulus to hepatic lipogenesis [22]. However, although 24-h C-peptide values were reduced with negative caloric balance, no difference was seen between the results for the soya and the milk treatments. Other factors than insulin secretion must be considered in order to explain the lower blood lipids seen on the soy diet.

Of interest is the lack of significant effect on blood lipids of modest weight reduction during the low caloric control and milk dietary periods. Large reductions in weight are well documented as being associated with reduced blood lipids [3]. The lack of conspicuous effect on lipids with smaller reductions in weight is consistent with findings where over 1 kg weight loss per month was recorded in hyperlipidemic individuals with no change in cholesterol or triglyceride levels [6].

The lack of change in blood lipids after weight loss seen in the present study may relate to the relatively low initial lipid values for the group (total cholesterol 5.1 ± 0.4 mmol/l, triglyceride 1.2 ± 0.2 mmol/l). No patient had serum cholesterol levels consistently over 5.9 mmol/l (although 2 had levels which ranged between 5.04 and 7.73 mmol/l); and none had triglyceride levels consistently above 2.0 mmol/l. Furthermore glucose tolerance status in the group was normal and all but one were normotensive. In relation to risk factors for cardiovascular disease, data on the metabolic effects of obesity indicate that the distribution of fat may be all important. Central or upper body obesity, (characterized by a high waist/hip ratio) as opposed to peripheral or lower body obesity has been associated with a wide range of metabolic abnormalities [23-27] including an increased risk of cardiovascular disease in both men [28] and women [29]. A high waist/hip ratio is associated with higher cholesterol and triglyceride levels, insulin resistance, raised FFA levels, carbohydrate intolerance [23-27,30] and raised blood pressure [31]. Although waist/hip ratios were not measured in our volunteers their relatively low blood lipids and absence of other

risk factors for cardiovascular disease in the presence of considerable obesity would suggest that in their case adiposity did not have a marked negative metabolic impact. Indeed, all our volunteers were women and it is therefore likely that the obesity tended to be of the gynoid or lower body type. In this situation weight reduction per se might not be expected to have major metabolic benefit.

On the milk and low calorie control diets the changes in serum cholesterol, albeit non-significant, were almost exactly accounted for by the small changes in weight, dietary saturated fat, polyunsaturated fat and cholesterol intakes. However, these factors accounted for only one third of the fall in serum cholesterol on the soy period. This provides strong supportive evidence for soy as the unaccounted for dietary variable responsible for this hypocholesterolemic effect.

The current studies demonstrate that consumption of a soy based liquid formula diet has a small but significant effect in reducing the total and LDL-cholesterol levels of overweight individuals with cholesterol levels within the normal range. Moderate weight loss on either a low calorie control or milk based diet in itself appeared to have no consistent effect on the level of either serum cholesterol or triglyceride.

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References

- 1 Sims, E.A.H., Goldman, R.F., Gluch, C.M., Horton, E.S., Kelleher, P.C. and Rowe, D.W., Experimental obesity in men, *Trans. Assoc. Am. Physicians*, 81 (1968) 153.
- 2 Albrink, M.J., Meigs, J.W. and Granoff, M.H., Weight gain and serum triglycerides in normal man. *N. Engl. J. Med.*, 26 (1962) 484.
- 3 Olefsky, J., Reaven, G.M. and Farquhar, J.W., Effects of weight reduction on obesity. Studies of high lipid and carbohydrate metabolism in normal and hyperlipoproteinemic subjects, *J. Clin. Invest.*, 53 (1974) 64.
- 4 Brownell, K.D. and Stunkard, A.J., Differential changes in plasma high-lipoprotein-cholesterol levels in obese men and women during weight reduction. *Arch. Intern. Med.*, 141 (1981) 1142.
- 5 Lewis, S., Haskell, W.L., Wood, P.D., Manoogian, N., Bailey, J.E. and Percira, M.B., Effects of physical activity on weight reduction in obese middle-aged women. *Am. J. Clin. Nutr.*, 29 (1976) 151.
- 6 Jenkins, D.J.A., Wolever, T.M.S., Kalmusky, J., Giudici, S., Giordano, C., Wong, G.S., Bird, J.N., Patten, R., Hall, M., Buckley, G. and Little, J.A., Low glycemic index carbohydrate foods in the management of hyperlipidemia, *Am. J. Clin. Nutr.*, 42 (1985) 604.
- 7 Kempner, W., Newborg, B.C., Peschel, R.L. and Skyler, J.S., Treatment of massive obesity with rice/reduction diet program, *Arch. Intern. Med.*, 135 (1975) 1575.
- 8 Sirtori, R., Agradi, E., Conti, F., Mantero, O. and Gatti, E., Soybean-protein diet in the treatment of type II hyperlipoproteinemia, *Lancet*, 1 (1977) 275.
- 9 Carroll, K.K., Giovannetti, P.M., Huff, M.W., Moase, O., Roberts, D.C.K. and Wolfe, B.M., Hypocholesterolemic effect of substituting soybean protein for animal protein in the diet of healthy young women, *Am. J. Clin. Nutr.*, 31 (1978) 1312.
- 10 Kritchevsky, D., Tepper, S.A. and Story, J.A., Influence of soy protein and casein on atherosclerosis in rabbits, *Fed. Proc.*, 37 (1978) 747.
- 11 Kritchevsky, D., Tepper, S.A., Czarniecki, S.K., Klurfeld, D.M. and Story, J.A., Experimental atherosclerosis in rabbits fed cholesterol-free diets. Part 9. Beef protein and texturized vegetable protein, *Atherosclerosis*, 39 (1981) 169.
- 12 Diem, K. and Lentner, C., *Documenta Geigy Scientific Tables*, J.R. Geigy SA, Basle, 1970, p. 712.
- 13 Paul, A.A. and Southgate, D.A.T., McCance and Widdowson's *The Composition of Foods*, Medical Research Council Report Series No. 297, 4th edn., HMSO, London, 1978.
- 14 Allain, C.C., Poon, L.S., Chan, C.S., Richmond, W. and Fu, P.C., Enzymatic determination of total serum cholesterol, *Clin. Chem.*, 20 (1974) 470.
- 15 Lopes-Virella, M.F., Stone, P., Ellis, S. and Colwell, J.A., Cholesterol determination in high-density lipoproteins separated by three different methods, *Clin. Chem.*, 23 (1977) 882.
- 16 Bucolo, G. and David, H., Quantitative determination of serum triglyceride by the use of enzymes, *Clin. Chem.*, 19 (1973) 476.
- 17 Friedwald, W.T., Levy, R.T. and Fredrickson, D.S., Estimation of the concentration of low density lipoprotein cholesterol in plasma without the use of the preparative ultracentrifuge, *Clin. Chem.*, 18 (1972) 499.
- 18 Kuzya, T., Matsuda, A., Saito, T. and Yoshida, S., Human C-peptide immunoreactivity (CPR) in blood and urine: evaluation of a radioimmuno-assay method and its clinical applications, *Diabetologia*, 12 (1976) 511.
- 19 Faulkner, W.R. and King, J.W., Renal function. In: Tietz, N.W. (Ed.), *Fundamentals of Clinical Chemistry*, W.B. Saunders Co., Philadelphia, PA, 1976, p. 995.
- 20 Reference deleted.
- 21 Gatti, E. and Sirtori, C.R., Soybean-protein and plasma cholesterol, *Lancet*, 1 (1977) 805.
- 22 Albrink, M.J., Newman, T. and Davidson, P.C., Effect of high and low fiber diets on plasma lipids and insulin, *Am. J. Clin. Nutr.*, 32 (1979) 1486.

- 23 Smith, U., Hammersten, J., Bjorntorp, P. and Kral, J., Regional differences and effect of weight reduction on human fat cell metabolism, *Eur. J. Clin. Invest.*, 9 (1979) 327.
- 24 Bjorntorp, P., Hazards in subgroups of human obesity, *Eur. J. Clin. Invest.*, 14 (1984) 239.
- 25 Smith, U., The adipose tissue and the metabolic complications of obesity. In: Bjorntorp, P., Vahouny, G.V. and Kritchevsky, D. (Eds.), *Dietary Fiber and Obesity*, Alan Liss, New York, 1985, pp. 33-39.
- 26 Kissebah, A.H., Characteristics of obese patients with hyperinsulinemia; the importance of body fat distribution. In: Bjorntorp, P., Vahouny, G.V., Kritchevsky, D. (Eds.), *Dietary Fiber and Obesity*, Alan Liss, New York, 1985, pp. 19-31.
- 27 Krotkiewski, M., Bjorntorp, P., Sjostrom, L. and Smith, U., Impact of obesity on metabolism in men and women: importance of regional adipose tissue distribution, *J. Clin. Invest.*, 72 (1983) 1150.
- 28 Larsson, B., Savrdsudd, K., Welin, L., Wilhelmsen, L., Bjorntorp, P. and Tibblin, G., Abdominal adipose tissue distribution, obesity and risk of cardiovascular disease and death. A 13 year follow-up study of men born in 1913, *Br. Med. J.*, 288 (1984) 1401.
- 29 Lapidus, L., Bengtsson, C., Larsson, B., Pennert, K., Rybo, E. and Sjorstrom, L., Adipose tissue distribution and risk of cardiovascular disease and death - 12 year follow up of participants in the population of women in Gothenburg, Sweden, *Br. Med. J.*, 289 (1984) 1257.
- 30 Hartz, A.J., Rupley, D.C. Jr., Kalkhoff, R.K. and Rimm, A.A., Relationship of obesity to diabetes: influence of obesity level and body fat distribution, *Prev. Med.*, 12 (1983) 351.
- 31 Blair, D., Habicht, J.P., Sims, E.A.H. et al., Evidence for an increased risk for hypertension with centrally located body fat and the effect of race and sex on this risk, *Am. J. Epidemiol.*, 119 (1984) 526.
- 32 Gordon, D.J., Saly, K.M., Roggenkamp, K.J. and Franklin, F.A., Jr., Dietary determinants of plasma cholesterol change in the recruitment phase of the Lipid Research Clinics Coronary Primary Prevention Trial, *Arteriosclerosis*, 2 (1982) 537.