# Substrate Utilization and Maximum Swimming Ability in Rats and Guinea Pigs Fed Wheat Germ Oil[1] (36712)

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Wheat germ oil has been reported to exert profound beneficial effects on humans, cattle, sheep, and laboratory animals (1), on the swimming performance of guinea pigs (2), and on human endurance, stamina and vigor (3–7). However, with the exception of the work of Alfin-Slater (8) on liver cholesterol content, essentially no studies have been reported on the beneficial effects of wheat germ oil as determined by modern biochemical techniques.

The data presented here were obtained on rats and guinea pigs fed diets containing approximately 20% protein, 5% fat, and 60% carbohydrate, plus salts and celluflour. The control (corn oil) diet contained all the nutrients and vitamins known to be present in wheat germ oil plus additional vitamins as required. In the experimental diet wheat germ oil (Viobin) was substituted for the

TABLE I. Composition of Diets.[a]

| Component | Composition (%) of control diets (g/100 g)[a] Diet: A | B | C |
|---|---|---|---|
| Corn oil[b] | 5.0 | 5.0 | 5.0 |
| Corn oil–vitamin E[c] | 1.0 | 1.0 | 1.0 |
| Corn oil–vitamins A and D[d] | 0.5 | 0.5 | 0.5 |
| Powdered whole milk[e] | 90.0 | — | — |
| Casein hydrolysate[f] | — | 18.0 | — |
| Lactalbumin hydrolysate[f] | — | 1.6 | — |
| Dextrin[g] | — | 19.3 | — |
| Corn starch[h] | — | 20.1 | 20.4 |
| Sucrose[i] | — | 20.1 | 10.0 |
| Celluflour[j] | — | 6.0 | 10.0 |
| Salt mix (Wesson modified Osborn-Mendel)[f] | 3.0 | 4.0 | 4.0 |
| Yeast extract[f] | — | 3.2 | — |
| D,L-Methionine[f] | — | 0.3 | — |
| Casein[f] | — | — | 30.0 |
| Dextrose[k] | — | — | 10.6 |
| Agar (type IV powder)[l] | 1.0 | — | 5.0 |
| Magnesium oxide[k] | — | — | 0.5 |
| Potassium acetate[k] | — | — | 2.5 |
| Vitamin–mineral mix[m] | 0.5 | 0.5 | 1.5 |

[a] For the *experimental* diets, 5% *wheat germ oil* (Viobin Corp., Monticello, IL) plus 1% pure corn oil were substituted for the 5% pure corn oil and the 1% corn oil–vitamin E supplement present in the control diets.

[b] Mazola Corn Products Co.

[c] Each gram of corn oil contained 12.5 mg *d*-alpha tocopherol acetate. Nutritional Biochemicals Corp., Cleveland, OH.

[d] Corn oil containing 1200 IU vitamin A and 220 IU vitamin D/g. The vitamins were obtained from White Laboratories, Inc., Kenilworth, NJ 07033.

[e] Challenge Cream and Butter Association, Berkeley, CA.

[f] General Biochemicals, Chagrin Falls, OH.

[g] Van Waters and Rogers, San Francisco, CA.

[h] Golden Eagle cornstarch, Cheney Bros., Oakland, CA.

[i] C and H cane sugar.

[j] Chicago Dietetic Supply, Inc., La Grange, IL.

[k] Mallinckrodt, St. Louis, MO.

[l] Sigma Chemical Co., St. Louis, MO.

[m] Cornstarch containing in each gram: 10.8 mg zinc sulfate, 124.6 mg ferrous sulfate, 9.8 mg cupric sulfate, 16.6 mg manganous chloride, 0.8 mg thiamine HCl, 1.0 mg riboflavin, 10.0 mg niacin, 0.5 mg pyridoxine HCl, 4.0 mg calcium pantothenate, 274 mg choline dihydrogen citrate, 200 mg inositol, 20 mg *p*-aminobenzoic acid, 0.4 mg folic acid, 0.04 mg biotin, 0.02 mg vitamin $B_{12}$, 0.4 mg menadione, 40 mg ascorbic acid.

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TABLE II. Influence of Wheat Germ Oil on Swimming Times in Rats and Guinea Pigs.[a]

| | Rats | | | | | | Guinea pigs | |
|---|---|---|---|---|---|---|---|---|
| Expt.: | 1 | | 2 | | 3 | | 4 | |
| Diet fed: | Control | WGO | Control | WGO | Control | WGO | Control | WGO |
| No. of animals | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 |
| No. of days fed | 33 | 33 | 28 | 28 | 33 | 33 | 35 | 35 |
| Food intake (g/day, av) | 11.0 | 10.6 | 17.2 | 17.2 | 19.7 | 19.7 | — | — |
| Av wt gain (g) | 116 | 93 | 125 | 121 | 137 | 138 | 179 | 168 |
| No. of swimming trials | 24 | 24 | 24 | 24 | 1 | 1 | 1 | 1 |
| Av swimming time (min) | 12.3 (±4.1) | 12.9 (±2.4) | 12.3 (±1.4) | 11.8 (±1.2) | 9.9 (±1.1) | 9.5 (±2.5) | 50.4 (±35) | 59.5 (±37) |

[a] For details concerning the diets, swimming trials and experimental designs, see text.

TABLE III. Incorporation of Various Substrates Into Tissue Total Lipids During *in Vitro* Incubation of Rat Liver and Heart Slices.[a]

| | | Corn oil diet | | | | Wheat germ oil diet | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Liver | | Heart | | Liver | | Heart | |
| Expt. no. | Substrate | Oxid | TL | Oxid | TL | Oxid | TL | Oxid | TL |
| 1 | Acetate-2-$^{14}$C | 11.2 | 1.9 | 25.7 | 0.3 | 13.6 | 1.6 | 29.4 | 0.4 |
| | Alanine-U-$^{14}$C | 14.2 | 0.08 | 1.3 | 0.02 | 13.6 | 0.07 | 1.4 | 0.03 |
| | Palmitate-1-$^{14}$C | 8.7 | 46.2 | 3.3 | 35.2 | 1.6 | 0.2 | 0.8 | 0.05 |
| | Glucose-U-$^{14}$C | 1.8 | 0.2 | 0.8 | 0.04 | 8.1 | 48.7 | 3.3 | 32.0 |
| 2 | Acetate-2-$^{14}$C | 10.1 | 4.8 | 26.8 | 0.09 | 11.1 | 3.8 | 25.9 | 0.09 |
| 3 | Acetate-2-$^{14}$C | 9.7 | 3.2 | 26.4 | 0.10 | 9.7 | 4.0 | 27.1 | 0.09 |

[a] The data are expressed as the percentage of radioactive substrate oxidized to $CO_2$ or incorporated into total lipid (TL) per 200 mg tissue during the 2 hr incubation at 37°.

corn oil. In contrast to previous reports, wheat germ oil did not influence swimming capacity or metabolism of various substrates by heart or liver.

*Materials and Methods. Animals.* Adult male Sprague-Dawley rats[3] weighing approximately 200 g at the start of each experiment were maintained in individual metabolism cages. Male guinea pigs[4] weighing 200–250 g at the start of the experiment were maintained in groups of 4 to 5 in cages with raised screen floors. All animals were fed their respective diets *ad libitum*. Water was always available.

[3] Horton Laboratories, Oakland, CA.

[4] ABC Caviary and Rabbitry, Pomona, CA.

*Experiment 1.* Two groups of 12 rats each were used. Body weights were about 200 g. The control group received Diet A, a whole milk diet as described by Sarett and Snipper (9) (Table I), whereas the experimental group received the modified Diet A containing 5% wheat germ oil (Footnote *a*, Table I). The rats were exercised to exhaustion 5 days/week for 4 weeks at which time they were sacrificed and the heart and liver used for *in vitro* incubation studies with various radioactive substrates.

*Experiment 2.* Similar to Expt 1 except that Diet B, a semipurified diet was used.

*Experiment 3.* The semipurified diet (Diet B) was used, but the rats were swum only

once after being on the diet for 33 days.

*Experiment 4.* Twelve guinea pigs were placed on a semipurified diet (Diet C, Table I) whereas 12 experimental guinea pigs received the modified Diet C containing 5% WGO (see footnote *a*, Table I). This diet was essentially the same as that used by Ershoff and Levin (2) and Ershoff (11). After feeding the diets for 4 weeks, the guinea pigs were swum once to exhaustion.

*Swimming trials.* Maximum swimming capacity of rats was tested by attaching a weight, equal to 5% of its body weight at the first trial, to the tail of the rat and placing it in a 24-gallon tank filled with water at 21–25° and allowing it to swim until he sank to a line 18 inches below the surface of the water. This technique has been described in detail by Kimeldorf and Baum (10). Guinea pigs were swum similarly except that weights were not used and the water temperature was maintained at 36–37°.

In vitro *studies and other analyses.* Rats were sacrificed by decapitation and tissues were rapidly removed, chilled, and slices were prepared. Tissue samples were incubated for 2 hr at 37° in 3 ml Krebs–Ringer bicarbonate buffer (14). The $^{14}CO_2$ produced was trapped in ethanolamine:2-ethoxyethanol (1:1). Lipids were extracted with chloroform–methanol (2:1) and a purified solution of total lipids was obtained. Aliquots of the purified lipid extracts were saponified with alcoholic–KOH and the nonsaponifiable, saponifiable and aqueous fractions were obtained. Measurements of $^{14}C$ in the $CO_2$, total lipids and the other fractions were made using a Packard Tri-Carb scintillation counter.

In some experiments, liver samples were quickly taken, digested with KOH and liver glycogen determined by the method of Roe (13). Serum cholesterol was measured according to Zlatkis and Boyle (12).

*Results and Discussion.* The data presented in Tables II–IV show that wheat germ oil had no demonstrable effect on the swimming capacity of rats or guinea pigs or on the metabolism of carbon-labeled acetate, alanine, glucose or palmitate by rat liver and heart slices. The results differ from previous-

TABLE IV. Distribution of $^{14}C$ in the Nonsaponifiable, Saponifiable and Aqueous Fractions Following Saponification of Total Lipids.[a]

| | | Rats fed corn oil diet | | | | | | Rats fed the wheat germ oil diet | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Liver | | | Heart | | | Liver | | | Heart | | |
| Expt. no. | Radioactive substrate | Nonsap | Sap | Aq | Nonsap | Sap | Aq | Nonsap | Sap | Aq | Nonsap | Sap | Aq |
| 1 | Acetate-2-$^{14}C$ | 4.2 | 63.1 | 32.7 | 0.9 | 43.1 | 56.0 | 7.6 | 55.8 | 36.6 | 0.8 | 46.2 | 53.0 |
| | Alanine-U-$^{14}C$ | 0.0 | 31.4 | 68.6 | 0.0 | 60.0 | 40.0 | 0.0 | 18.5 | 81.5 | 3.0 | 72.7 | 24.3 |
| | Glucose-U-$^{14}C$ | 1.1 | 8.7 | 90.2 | 0.7 | 13.1 | 86.2 | 0.3 | 8.8 | 90.9 | 0.7 | 16.1 | 83.2 |
| | Palmitate-1-$^{14}C$ | 2.1 | 86.3 | 11.6 | 1.8 | 87.7 | 10.5 | 1.7 | 91.0 | 7.3 | 1.8 | 89.4 | 8.8 |
| 2 | Acetate-2-$^{14}C$ | 8.0 | 80.8 | 11.2 | 10.0 | 68.0 | 22.0 | 14.6 | 70.7 | 14.7 | 9.8 | 62.0 | 28.2 |
| 3 | Acetate-2-$^{14}C$ | 12.4 | 68.2 | 19.4 | 10.4 | 55.4 | 34.2 | 11.2 | 78.2 | 10.6 | 11.7 | 56.7 | 31.6 |

[a] The data are expressed as the percentage of total lipid-$^{14}C$ recovered in the nonsaponifiable, saponifiable, and aqueous soluble fractions after saponification.

ly published reports. The reasons for the differences are not apparent. It should be noted, however, that in the present report two different diets were used with rats and the treatment of guinea pigs was essentially that described by Ershoff and Levin (2).

No differences were found in liver glycogen or serum cholesterol levels between the rats fed corn oil vs those fed wheat germ oil (Expts 1 and 2).

Thus, the beneficial effects of wheat germ oil on stamina and metabolism were not corroborated in the present studies despite the relatively large amounts of wheat germ oil used. It is concluded that previously claimed beneficial effects of wheat germ oil on stamina and metabolism in experimental animals must be reexamined carefully. How these studies relate to reported beneficial effects of wheat germ oil on human stamina and performance is, of course, not known.

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