Insulin Secretion in the Premature Infant

Response to Glucose and Amino Acids

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SUMMARY

Serum insulin was measured in fifty-one premature infants in the first twenty-four hours of life following the administration of glucose, a mixture of amino acids, or glucose with an amino acid mixture. Infusion of glucose (1.25 gm.) caused a rapid increase of blood glucose and a small rise in serum insulin. The amino acid mixture (2.5 gm.) produced, on the contrary, a rapid and marked increase of serum insulin but only a slight rise of blood glucose. When the dose of the amino acid mixture was halved, rise of serum insulin and blood glucose was small. Combining the reduced dose of amino acid mixture with glucose produced a rapid and striking increase of both serum insulin and blood glucose. DIABETES *22*:349-53, May, 1973.

Recent evidence indicates that glucose is a primary metabolic fuel in the fetus[1] and that insulin can be detected in the human fetal pancreas and blood at eight to ten weeks of gestation.[2-4] Little is known about control of insulin secretion during fetal and neonatal life. Whereas intravenously administered glucose causes a sluggish and delayed insulin rise in normal newborns and premature infants,[5,6] intravenous administration of a mixture of amino acids or arginine to premature infants produces a prompt and marked release of insulin.[7,8]

It has been shown that in the adult, glucose and amino acids are synergistic in their insulin stimulatory action.[9] The studies to be described were performed in premature infants to investigate changes in the concentrations of serum insulin following the administration of a mixture of amino acids with and without the addition of glucose.

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MATERIALS AND METHODS

Fifty-one vaginally delivered infants judged premature on the basis of clinical examination and gestational age[10-12] were studied. Their mean gestational age was 35 ± 2 weeks and their weights ranged from 1,500 to 2,580 gm. with a mean weight of 2,165 ± 46 (S.E.M.). The infants were kept warm in incubators. At times varying from three to twenty-four hours after birth, before feeding was initiated, a polyethylene catheter was inserted in the umbilical vein to withdraw blood samples and administer infusions.

Serial levels of serum insulin, blood glucose, and α-amino nitrogen were measured after administration of one of the following substances: [1] Glucose (1.25 gm.) as a 10 per cent solution; [2] A mixture of amino acids (2.5 or 1.25 gm.) as a 10 per cent solution; or [3] Glucose plus a mixture of amino acids, each in amounts of 1.25 gm. The solutions were administered by a constant infusion pump over a period of thirty minutes. Blood samples were obtained at 0, 30, 60, and 120 minutes.

L-amino acids (Carlo Erba, Milano, Italy) were obtained in crystalline form as pure amino acids, with the exception of arginine, histidine, and lysine, which were obtained as hydrochlorides. A 2.5 gm. quantity of the mixture was composed of 330 mg. arginine, 380 mg. lysine, 330 mg. phenylalanine, 390 mg. leucine, 240 mg. methionine, 300 mg. valine, 120 mg. histidine, 230 mg. isoleucine, and 180 mg. threonine. The mixture was made up in 25 ml. normal saline.

Blood was collected for glucose determination in a tube containing sodium fluoride. Glucose was measured by a glucose oxidase method (Sigma Chemical Co., St. Louis). For insulin determination, a separate blood sample was allowed to clot in a refrigerator at 4° C., after which the serum was separated and frozen. Insulin was determined in alcohol extracts of serum[13] by the immunochemical method of Hales and Randle[14] using antisera (Burroughs Wellcome) and I-125-insulin

(Radiochemical Center, Amersham, England). Human insulin was used as a standard. The method was sensitive to 1 μU. of insulin per milliliter of serum. All samples from the same infant were determined in triplicate in the same assay.

Concentrations of plasma α-amino nitrogen were determined by the technic of Matthews, Muir, and Baron.[15]

RESULTS

Glucose infusion produced a small increase of serum insulin in eleven premature infants with mean body weight of 2,226 gm. ± 82 gm. S.E.M. Serum insulin went from 10 ± 1 μU. per milliliter at 0 minutes to 17 ± 2 μU. per milliliter at 30 minutes and 19 ± 3 μU. per milliliter at 60 minutes; the reading decreased to 14 ± 3 μU. at 120 minutes. Mean blood glucose increased rapidly from a control value of 37 ± 2 mg. to 156 ± 6 mg. per 100 ml. at 30 minutes and then fell to 98 ± 6 mg. and 68 ± 7 mg. per 100 ml. at 60 and 120 minutes, respectively (figure 1).

In fifteen premature infants with mean body weight of 2,123 ± 109 gm., infusion of a solution containing 2.5 gm. of a mixture of amino acids promptly augmented serum insulin in all subjects. Mean serum insulin rose from a control value of 14 ± 2 μU. per milliliter to a peak of 100 ± 16 μU. per milliliter at 30 minutes. It declined to 87 ± 26 μU. per milliliter at 60 minutes but was still well above control level at the end of the experiment in all subjects but one (mean 39 ± 7 μU. per milliliter). Maximal increase occurred at 30 minutes in five subjects and at 60 minutes in eight others. Mean plasma α-amino nitrogen rose from a control level of 5 ± 0.3 mg. to 34 ± 2 mg. per 100 ml. at 30 minutes, declined to 23 ± 1 mg. and 12 ± 0.8 mg. per 100 ml. at 60 and 120 minutes, respectively. Mean blood glucose increased gradually from a control value of 36 ± 4 mg. to 73 ± 4 mg. per 100 ml. at 120 minutes (figure 2).

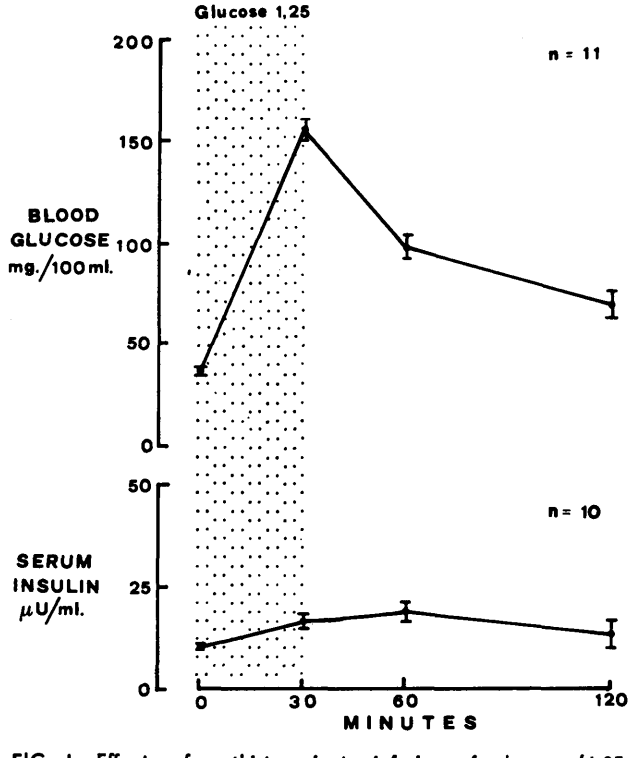

FIG. 1. Effects of a thirty minute infusion of glucose (1.25 gm.) on blood glucose and serum insulin levels (± S.E.M.) in premature infants.

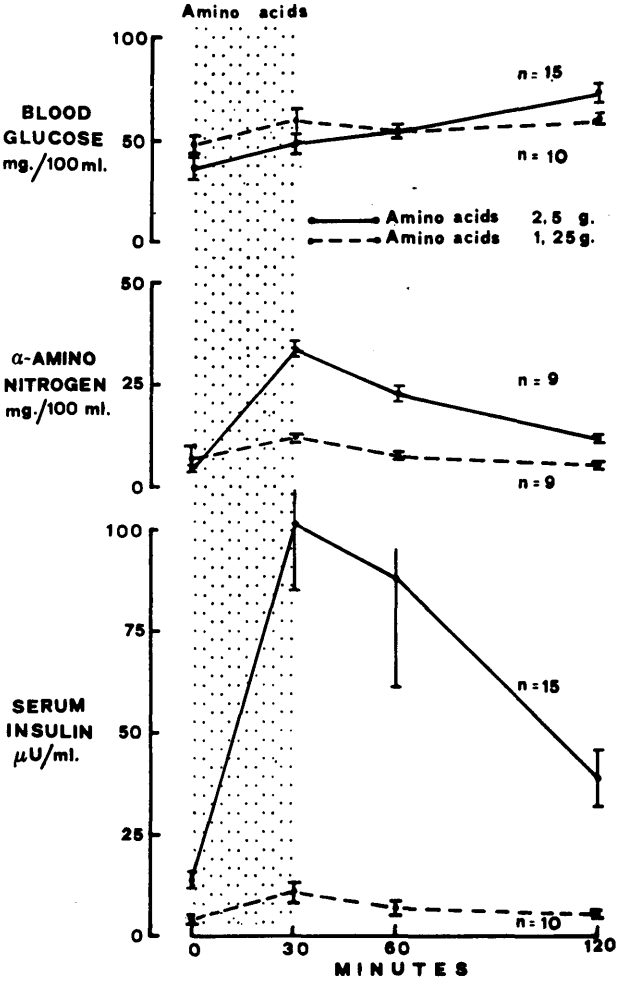

FIG. 2. Comparison of blood glucose, plasma α-amino nitrogen, and serum insulin levels (± S.E.M.) in premature infants following a thirty minute infusion of a 2.5 gm. or 1.25 gm. mixture of amino acids.

The amino acid mixture in a reduced amount of 1.25 gm. was given to ten premature infants with mean body weight of 2,168 ± 87 gm.; serum insulin rose slightly from 4 ± 2 μU. per milliliter to 11 ± 3 μU. per milliliter after 30 minutes and then fell to 7 ± 2 μU. per milliliter at 60 minutes and 5 ± 1 μU. per milliliter at 120 minutes. Mean plasma α-amino nitrogen rose from a control level of 7 ± 3 mg. to 12 ± 1 mg. per 100 ml. at 30 minutes and then declined to 8 ± 0.5 mg. and 6 ± 0.5 mg. per 100 ml. at 60 and 120 minutes, respectively. Mean blood glucose rose from a control value of 48 ± 4 mg. to 60 ± 3 mg. per 100 ml. at 120 minutes (figure 2).

In fourteen premature infants with mean body weight of 2,094 ± 61 gm., infusion of glucose with 1.25 gm. of amino acid mixture increased serum insulin rapidly and markedly from 9 ± 0.9 μU. per milliliter to a peak of 75 ± 21 μU. per milliliter at 30 minutes. The value was still 73 ± 13 μU. per milliliter at 60 minutes and then declined to 37 ± 6 μU. per milliliter at 120 minutes. Maximal increase occurred at 30 minutes in five subjects and at 60 minutes in eight others. At the same time, this infusion caused a hyperglycemia similar to that of glucose alone and also an increase of plasma α-amino nitrogen similar to that of 1.25 gm. of amino acid mixture alone (figure 3).

DISCUSSION

The study indicates that in premature infants 2.5 gm. of a mixture of amino acids produces a large rise in serum insulin and that administration of the amino acid mixture, in a lower dose, with glucose increases serum insulin even more than does infusion of the substances separately. Following the administration of glucose alone, the insulin response was small, as has been shown by others.[6-8,16-19] An over-all low response to glucose has also been documented in vitro with human fetal pancreas[20] and pancreas of fetuses or newborns of other species, such as monkeys, sheep, and rats.[21-24]

The explanation for the low insulin response to glucose remains uncertain. Recent investigations have suggested that insulin release may be dependent on intracellular accumulation of cyclic 3′,5′ AMP within the beta cell.[25,26] Therefore, the low effectiveness of glucose in the fetal pancreas may be due to inadequate intracellular accumulation of the cyclic nucleotide. Theophylline, an agent known to increase cyclic AMP in the beta cells,[25] stimulates insulin release in the premature infant and monkey fetus[27,28] and in fetal rat explants.[28]

The in vitro studies of glucose metabolism by Heinze and Steinke show a marked difference between fetal and adult islets.[29] Their findings suggest a metabolic explanation for the difference in glucose-induced insulin release.

Decreased insulin synthesis is not a likely explanation for this phenomenon, since a rapid rise of serum insulin level is evoked by a large dose of amino acids and glucose with amino acids. A possible explanation for this peculiar insulin response of premature infants could be their low blood sugar. In vitro studies[30] have

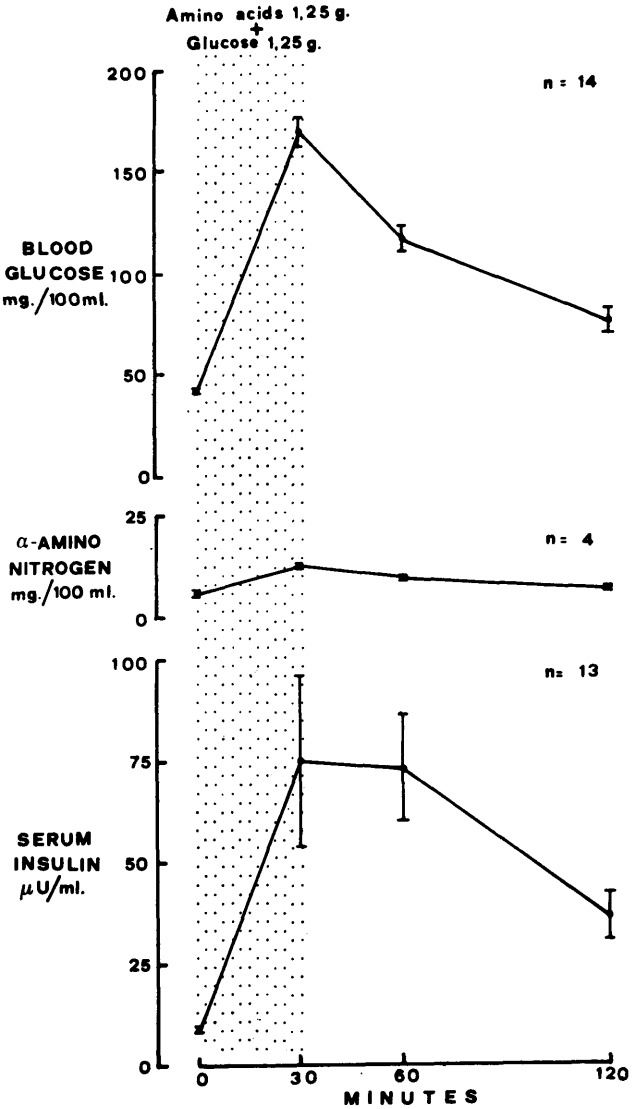

FIG. 3. Blood glucose, α-amino nitrogen, and serum insulin levels (± S.E.M.) following a thirty minute infusion of a mixture of amino acids (1.25 gm.) and glucose (1.25 gm.).

shown that stimulation of insulin secretion by glucose usually requires a minimum concentration of glucose above 50 mg. per 100 ml.

The mechanism of amino acids initiating insulin release in man is unknown. Evidently, the insulinotropic mechanisms of glucose and of amino acids differ.

Recently King et al. demonstrated an attenuated but statistically significant increase of plasma insulin in the newborn after intravenous arginine infusion.[31] Chez et al. infused monkey fetuses with a mixture of amino acids equal to that used by us and obtained results similar to ours in premature infants.[31] They also noted that fetuses of diabetic mothers required a tenfold lower concentration of amino acids for a similar response.

The extent to which insulin influences metabolism in the newborn remains to be determined. Our data could be said to suggest that the principal function of insulin in the premature infant is to stimulate utilization of amino acids for synthesis of protein during a time of very active growth.

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REFERENCES

[1] Cornblath, M., and Schwartz, R.: Disorders of carbohydrate metabolism in infancy. Major problems in clinical pediatrics. Vol. III. Philadelphia, W. B. Saunders, 1966.

[2] Steinke, J., and Driscoll, S. G.: The extractable insulin content of pancreas from fetuses and infants of diabetic and control mothers. Diabetes *14*:573, 1965.

[3] Adam, P. A. J., Teramo, K., Räihä, N., Gitlin, D., and Schwartz, R.: Human fetal insulin metabolism early in gestation: response to acute elevation of the fetal glucose concentration and placental transfer of human insulin-I-131. Diabetes *18*:409, 1969.

[4] Rastogi, G. K., Letarte, J., and Fraser, T. R.: Immunoreactive insulin content of 203 pancreases from foetuses of healthy mothers. Diabetologia 6:445, 1970.

[5] Jørgensen, K. R., Deckert, T., Pedersen, L. M., and Pedersen, J.: Insulin, insulin antibody and glucose in plasma of newborn infants of diabetic women. Acta Endocrinol. *52*: 154, 1966.

[6] Spellacy, W. N., Gall, S. A., and Carlson, K. L.: Carbohydrate metabolism of the normal term newborn: plasma insulin and blood glucose levels during an intravenous glucose tolerance. Obstet. Gynecol. *30*:580, 1967.

[7] Grasso, S., Saporito, N., Messina, A., and Reitano, G.: Serum insulin response to glucose and amino acids in the premature infant. Lancet *2*:755, 1968.

[8] Reitano, G., Grasso, S., Distefano, G., and Messina, A.: The serum insulin and growth hormone response to arginine and arginine with glucose in the premature infant. J. Clin. Endocrinol. Metab. *33*:924, 1971.

[9] Floyd, J. C., Jr., Fajans, S. S., Pek, S., Thiffault, C. A., Knopf, R. F., and Conn, J. W.: Synergistic effect of essential amino acids and glucose upon insulin secretion in man. Diabetes *19*:109, 1970.

[10] American Academy of Pediatrics. Committee on fetus and newborn nomenclature for duration of gestation, birth weight and intrauterine growth. Pediatrics *39*:935, 1967.

[11] Battaglia, F. C., and Lubchenco, L. O.: A practical classification of newborn infants by weight and gestational age. J. Pediatr. *71*:159, 1967.

[12] Yerushalmy, J.: The classification of newborn infants by birth weight and gestational age. J. Pediatr. *71*:164, 1967.

[13] Grodsky, G. M., and Forsham, P. H.: An immunochemical assay of total extractable insulin in man. J. Clin. Invest. *39*:1070, 1960.

[14] Hales, C. N., and Randle, P. J.: Immunoassay of insulin with insulin-antibody precipitate. Biochem. J. *88*:137, 1963.

[15] Matthews, D. M., Muir, G. G., and Baron, D. N.: Estimation of alpha-amino nitrogen in plasma and urine by the colorimetric ninhydrine reaction. J. Clin. Pathol. *17*:150, 1964.

[16] Milner, R. D. G., and Hales, C. N.: Effect of intravenous glucose on concentration of insulin in maternal and umbilical cord plasma. Br. Med. J. *1*:284, 1965.

[17] Thomas, K., de Gasparo, M., and Hoet, J. J.: Insulin levels in the umbilical vein and in the umbilical artery of newborn of normal and gestational diabetic mothers. Diabetologia *3*:299, 1967.

[18] Tobin, J. D., Roux, J. F., and Soeldner, J. S.: Human fetal insulin response after acute maternal glucose administration during labor. Pediatrics *44*:668, 1969.

[19] Gentz, J. C. H., Warrner, R., Persson, B. E. H., and Cornblath, M.: Intravenous glucose tolerance, plasma insulin, free fatty acids and beta-hydroxybutyrate in underweight newborn infants. Acta Pediatr. Scand. *58*:490, 1969.

[20] Espinosa de los Monteros, A., Driscoll, S. G., and Steinke, J.: Insulin release from isolated human fetal pancreatic islets. Science *168*:1111, 1970.

[21] Mintz, D. H., Chez, R. A., and Horger, III, E. O.: Fetal insulin and growth hormone metabolism in the subhuman primate. J. Clin. Invest. *48*:176, 1969.

[22] Bassett, J. M., and Thorburn, G. D.: The regulation of insulin secretion by the ovine foetus in utero. J. Endocrinol. *50*:59, 1971.

[23] Lambert, A. E., Jeanrenaud, B., and Renold, A. E.: Enhancement by caffeine of glucagon-induced and tolbutamide-induced insulin release from isolated foetal pancreatic tissue. Lancet *1*:819, 1967.

[24] Asplund, K., Westman, S., and Hellerström, C.: Glucose stimulation of insulin secretion from the isolated pancreas of foetal and newborn rats. Diabetologia *5*:260, 1969.

[25] Turtle, J. R., and Kipnis, D. M.: An adrenergic receptor mechanism for the control of cyclic 3′,5′ adenosine monophosphate synthesis in tissues. Biochem. Biophys. Res. Commun. *28*:797, 1967.

[26] Malaisse, W. J., Malaisse-Lagae, F., and Mayhew, D.: A possible role for the adenylcyclase system in insulin secretion. J. Clin. Invest. *46*:1724, 1967.

[27] Grasso, S., Messina, A., Saporito, N., and Reitano, G.: Effect of theophylline, glucagon and theophylline plus glucagon on insulin secretion in the premature infant. Diabetes *19*:837, 1970.

[28] Chez, R. A., Mintz, D. H., and Hutchinson, D. L.: Effect of theophylline on glucagon and glucose-mediated plasma insulin response in subhuman primate fetus and neonate. Metabolism *20*:805, 1971.

[29] Heinze, E., and Steinke, J.: Glucose metabolism of isolated pancreatic islets: difference between fetal, newborn and adult rats. Endocrinology *88*:1259, 1971.

[30] Grodsky, G. M., Batts, A. A., Bennett, L. L., Vcella, C., McWilliams, N. B., and Smith, D. F.: Effects of carbohydrates on secretion of insulin from isolated rat pancreas. Am. J. Physiol. *205*:638, 1963.

[31] King, K. G., Butt, J., Raivio, K., Räihä, N., Roux, J., Teramo, K., Yamaguchi, K., and Schwartz, R.: Human maternal and fetal insulin response to arginine. N. Engl. J. Med. *285*:607, 1971.

[32] Mintz, D. H., Chez, R. A., and Hutchinson, D. L.: Subhuman primate pregnancy complicated by streptozotocin induced diabetes mellitus. J. Clin. Invest. *51*:847, 1972.

Dietary Carbohydrate and Postprandial Hyperlipemia

When the dietary carbohydrate is sucrose rather than glucose the postprandial alimentary hyperlipemia persists for a long time. This is related to the lower levels of blood glucose and serum insulin.

The relationship of diet to obliterative arterial disease continues to be the subject of investigation, and several large studies have been mounted to determine the possible beneficial effects of various dietary changes. There seems to be little doubt now that diets high in saturated fats and cholesterol predispose to hypercholesteremia, and it is also clear that hypercholesteremia is closely related to atheroma production. Several of these relevant studies have previously been reviewed (Nutrition Reviews *20*:41, 1962; *24*:228, 1966; *28*:228, 1970).

The tendency in all these investigations has been to concentrate on serum levels of cholesterol as being the most important of the lipid fractions which could contribute to atheroma formation. Other fractions such as the serum triglycerides have been held to be relatively unimportant since, as has been pointed out previously, Type I hyperlipemia, which is associated with high serum triglyceride levels and normal serum cholesterol, does not seem to be associated with a higher incidence of occlusive arterial disease early in life. Conversely, in familial hypercholesteremia (Type II hyperlipemia), where the triglyceride levels are relatively normal, there is a very high risk of occlusive disease, and myocardial infarction may occur even in children (H. Malmros, Lancet *1*:94, 1970). Nevertheless there are suggestions that high triglyceride levels may be a relevant factor in the production of atheroma (M. J. Albrink, J. W. Meigs, and E. B. Man, Am. J. Med. *31*:4, 1961). M. A. Denborough (Clin. Sci. *25*:115, 1963) found that in twenty male patients who had had myocardial infarctions, the fasting serum triglycerides were higher and there was a greater degree of postprandial hyperlipemia than in controls. Since the betalipoproteins contain both cholesterol and triglyceride, some association between these two fractions would be expected.

A considerable number of investigators have found that diets which are very high in sucrose result in elevated levels of serum triglycerides when compared with similar diets containing starch (for example, I. Macdonald, Clin. Sci. *29*:193, 1965; M. A. Antar and M. A. Ohlson, J. Nutrition *85*:329, 1965). In some studies a consistent hypertriglyceridemic response to sucrose feeding was only obtained when the diet contained saturated fat as well (B. L. Birchwood et al., Atherosclerosis *11*:183, 1970; Antar et al., Ibid. *11*:184, 1970), indicating some synergistic action between saturated fats and sucrose. In a recent study, J. I. Mann, A. S. Truswell, and B. L. Pimstone (Clin. Sci. *41*:123, 1971) compared the effects of sucrose and glucose on the postprandial hyperlipemia in two groups of subjects: middle-aged men (thirty to fifty-eight years) and younger men (twenty to twenty-five years). Both groups received two formula breakfasts in which the fat was sunflower-seed oil, but the source of carbohydrate was either glucose or sucrose.

In the middle-aged men there was an increase of

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