Persistent Impairment of Insulin Secretory Response to Glucose in Adult Rats After Limited Period of Protein-Calorie Malnutrition Early in Life

INGEMAR SWENNE, CAROLINE J. CRACE, AND R. DAVID G. MILNER

SUMMARY

The effect of a limited period of protein-calorie malnutrition in young rats on glucose tolerance, insulin secretory response to glucose, and tissue composition in the adult was studied. Three-week-old rats were weaned onto semisynthetic diets containing either 5% protein (low protein; LP) or 15% protein (control; C) and maintained for 3 wk on their respective diets. At 6 wk of age all rats were returned to a commercial rat chow diet (18% protein). Glucose tolerance, insulin secretory response to glucose, and the protein/DNA ratio in liver, skeletal muscle, heart, kidney, small intestine, and lung were investigated at 3, 6, and 12 wk of age. Rats receiving LP diet failed to gain weight, but growth resumed immediately when they were transferred to commercial rat chow. They did not, however, catch up with C rats. Glucose tolerance and insulin secretory response to glucose remained similar between 3 and 12 wk in C rats. In 6-wk-old LP rats, glucose tolerance was impaired, and the insulin secretory response to glucose was absent. At 12 wk of age the glucose tolerance of the LP rats had normalized, but the insulin secretory response was still blunted. In 6-wk-old LP rats there was an inhibition of the age-dependent increase in cell size, shown by lowered protein/DNA ratios in all tissues studied. This decrease in cell size persisted at 12 wk in liver, skeletal muscle, heart, and lung.

We conclude that protein-calorie malnutrition early in life persistently impairs the insulin secretion. The persistently lowered protein/DNA ratios in many tissues may be related to this lowered capacity for insulin secretion. The individual could have an impaired ability to respond to diabetogenic and nutritional challenges, and it is thus possible that the early malnutrition may predispose for diabetes. *Diabetes* 36:454–58, 1987

From the Department of Paediatrics, University of Sheffield, Children's Hospital, Sheffield, United Kingdom.

Address correspondence and reprint requests to Dr. I. Swenne, Dept. of Medical Cell Biology, BMC, Box 571, S-751 23 Uppsala, Sweden.

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The relationship between overnutrition, obesity, and diabetes is well recognized, but the opposite, malnutrition, is a less-studied cause of the disease. There is, however, a high prevalence of malnutrition-related diabetic syndromes in many developing countries (1). The patients develop these types of diabetes in young adulthood and have the stigmata and history of previous and/or present malnutrition (reviewed in refs. 2–4). The precise etiology is yet to be elucidated, but there is a strong association with malnutrition, and more specifically protein deficiency, in geographically widely separated areas (4). Thus, the possibility that malnutrition in early infancy and childhood could result in partial failure of the β-cell function and clinical onset of diabetes later in life merits further study (1).

Kwashiorkor, the syndrome of protein deficiency in childhood, is characterized by, among other things, impaired glucose tolerance and absent or blunted insulin secretory response to glucose (5–16). The development of this disturbance seems to depend more on protein deficiency than on calorie deficiency, because infants with marasmus tend to have normal or only slightly impaired glucose tolerance and insulin secretion (6,8,10,12,13). Treatment of kwashiorkor with a diet adequate in protein and other nutrients results in a partial recovery of glucose tolerance and insulin secretion within weeks (7–14), but conflicting data have been presented as to whether the impairment persists later in life (10,17–19). These studies are difficult to interpret because nutrition and health during the years after recovery from kwashiorkor may influence the final outcome, especially in developing countries where nutrition may at best be only marginally adequate.

With experimental models of protein-calorie malnutrition it has been possible to reproduce the impaired glucose tolerance and decreased insulin secretory response to glucose of human kwashiorkor (20–24). However, the possibility that these defects may persist after a period of adequate nutrition

TABLE 1
Composition of the experimental diets (g/100 g)

Ingredients	Low-protein diet	Control diet
Casein	5	15
Rice starch	81	71
Cellulose	2.6	2.6
Corn oil	4.8	4.8
DL-Methionine	0.01	0.03
Solutions of vitamins, minerals, and trace elements*	~100	~100

*For detailed composition see ref. 25.

and influence growth and development of the animal has not been studied. We fed weanling rats a diet insufficient in protein for a limited period and demonstrated a retained diminished insulin secretory response to glucose after the rats were returned to an adequate diet.

MATERIALS AND METHODS

Diets. The pelleted semisynthetic experimental diets were made as previously described (25) and contained 5% protein (low-protein diet; LP) or 15% protein (control diet; C) and were generously provided by Dr. R. D. E. Rumsey (Subdepartment of Human Gastrointestinal Physiology and Nutrition, Univ. of Sheffield, Sheffield, UK). Their compositions are shown in Table 1. Methionine, the first limiting amino acid of casein, was added to obviate acute deficiencies of this amino acid. The loss in energy due to the reduction in protein content of the LP diet was compensated for by increasing the carbohydrate content. The two diets were thus isoenergetic and, apart from their protein content, identical in all respects.

The commercially available rat chow used in the protocol (CRM, Labsure, Greeff, Croydon, UK) contained 18.1% (wt/vol) crude protein, 2.4% oil, 3.6% fiber, and 57.0% carbohydrates.

Animals. Young male and female Wistar rats were obtained from a local breeding colony (Univ. of Sheffield). The animals had free access to CRM rat chow and tap water throughout breeding, pregnancy, and lactation. Mating was performed by caging males with females overnight, and pregnancy was confirmed by the presence of vaginal plugs the following morning. The pregnant rats were caged individually, and delivery took place on day 22 of gestation. The day after delivery the offspring were counted, and large litters were reduced to 10 pups. Litters with <6 pups were not used. At 21 days of age the young rats were weighed, caged in groups of 3 of the same sex, and weaned onto LP or C diet. The two diets were fed ad libitum for 3 wk when both groups of rats (denoted LP rats and C rats), at 6 wk of age, were returned to CRM diet until the end of the experiment, 12 wk of age. The animals were weighed weekly except during the 1st wk on CRM diet, when they were weighed daily.

Glucose tolerance and insulin secretory response to glucose. An intraperitoneal glucose tolerance test (GTT) was performed at 3, 6, and 12 wk of age in LP and C rats of both sexes. The rats were starved overnight (15–17 h) before the test. They were then injected intraperitoneally with a 30% (wt/vol) glucose solution at a dose of 2 g glucose/kg body wt. In the 12-wk-old rats, blood samples were obtained from the cut tip of the tail immediately before and 30, 60, and 120 min after the injection. To obtain sufficient amounts of blood, 3- and 6-wk-old rats were killed by cervical dislocation at the different time points, and blood was collected from the cut cervical vessels. The blood samples were allowed to clot and were centrifuged. The serum was separated and stored frozen at −20°C until assayed. Glucose was measured with a Yellow Springs glucose analyzer (Clandon, Aldershot, Hampshire, UK). Insulin was measured with a double-antibody immunoassay system (Wellcome, Temple Hill, UK) and rat insulin standard (Novo, Copenhagen, Denmark).

Tissue composition. At the end of the GTT, all rats were killed, and small tissue samples of cardiac muscle, lung, liver, small intestine, kidney cortex, and skeletal muscle (quadriceps femoris) were obtained. The samples were finely chopped up in distilled water and disrupted ultrasonically. The protein (26) and DNA (27,28) content was measured in the sonicates, and the protein/DNA ratio was calculated and used as an index of the cell size.

Statistical analyses. Differences in body weight, glucose tolerance, insulin response, and tissue composition between C and LP rats of the same age were analyzed with Student's two-tailed t test for unpaired observations. Age trends in tissue composition were evaluated by analysis of variance.

RESULTS

Body weight gain. When the rats were weaned, C rats of both sexes rapidly gained weight and continued to grow throughout the observation period (Fig. 1). There was a small increase in the rate of weight gain when the C rats were transferred to CRM diet at 6 wk of age, suggesting a higher nutritional efficiency of this diet. Indeed, rats in the departmental colony weaned on CRM diet initially gained weight more rapidly than C rats (C.J.C., unpublished observations). However, C rats caught up, and from 7 wk of age onward, their body weight was similar to that of rats weaned on CRM diet.

LP rats gained little weight from weaning to 6 wk of age. During this period, LP rats reduced their food intake and consumed only ~35% of the food compared with C rats. When transferred to CRM diet, they immediately resumed growth and gained weight at a rate similar to that of C rats. The LP rats thus never caught up in weight but remained smaller than the C rats throughout the experiment.

Glucose tolerance and insulin secretory response to glucose. Glucose tolerance and insulin secretory response to the intraperitoneal glucose load was similar in male and female rats, and the results were consequently pooled. In C rats of the different age groups the serum glucose values increased to a peak of 8–10 mM 30 min after the glucose injection and subsequently declined to values around 5 mM at 60 and 120 min. The peak insulin response at 30 min reached values >3 ng/ml in all age groups and returned to fasting levels at the end of the test (Fig. 2). The insulin/glucose ratio at 30 min, an index of the sensitivity of the β-cell to glucose, was 0.46 ± 0.06 ng/mmol (mean ± SE; n = 25) in 3-wk-old rats, 0.42 ± 0.03 ng/mmol (n = 25) in 6-wk-old C rats, and 0.33 ± 0.02 ng/mmol (n = 39) in 12-wk-old C rats.

At 6 wk of age, LP rats had an impaired glucose tolerance,

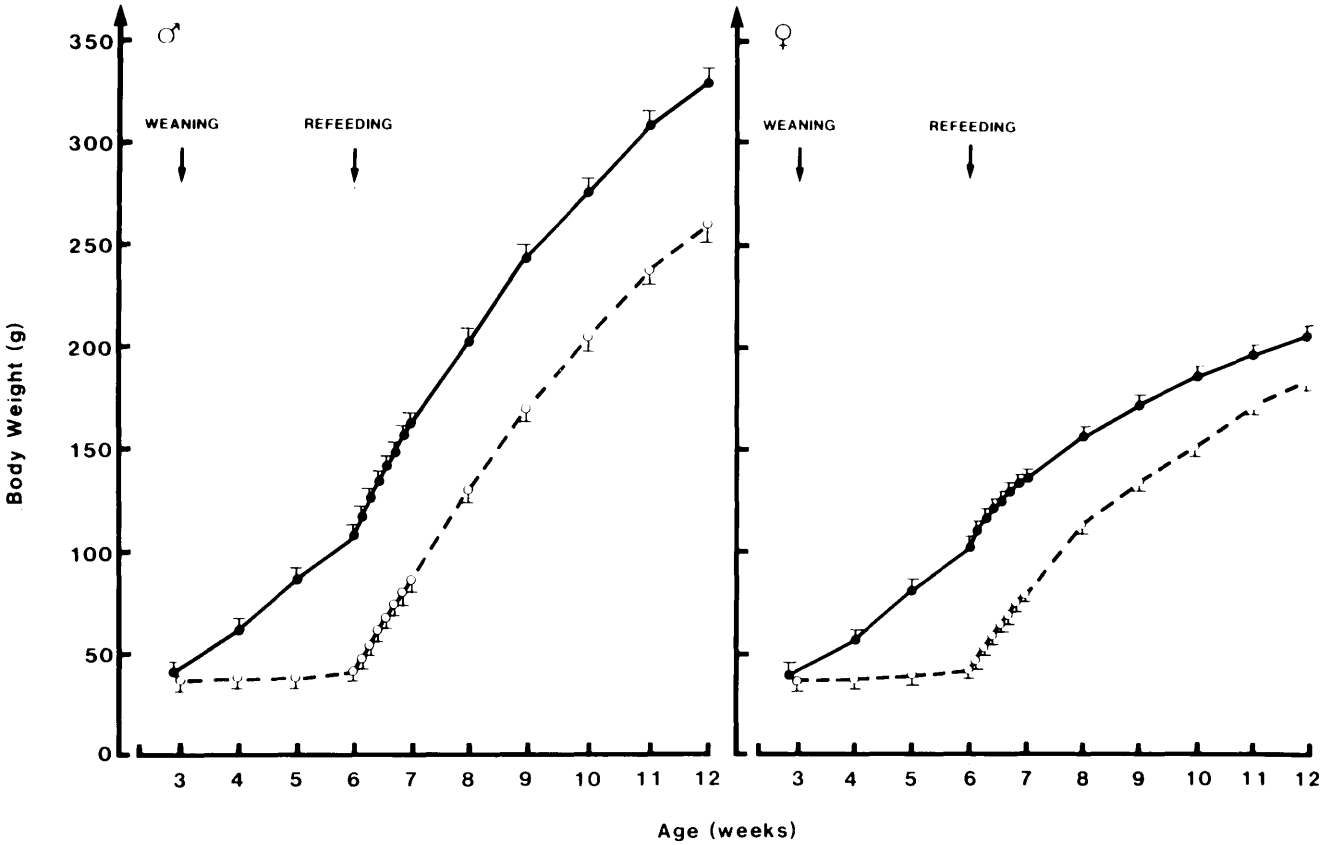

FIG. 1. Growth curves of rats fed diets with different protein content between 3 and 6 wk of age. Male (*left panel*) and female (*right panel*) Wistar rats were weaned at 3 wk of age onto control diet (15% protein; ●) or low-protein diet (5% protein; ○) and maintained ad libitum on respective diets for 3 wk. At 6 wk of age they were returned to commercial rat chow (protein content 18.1%) for remainder of experiment. Values are means ± SE for 18–22 rats.

with peak values approaching 15 mM. The glucose levels, however, rapidly returned to normal and were not different from those of C rats at 120 min. The fasting insulin levels of 6-wk-old LP rats were lower than those of the C rats. The LP rats did not exhibit a significant insulin secretory response to glucose, and their serum insulin levels remained lower than those of the C rats throughout the test. In the 6-wk-old LP rats the insulin/glucose ratio at 30 min was 0.07 ± 0.006 ng/mmol (n = 28; $P < .001$ vs. 6-wk-old C rats).

At 12 wk of age the glucose tolerance of the LP rats had normalized and did not differ from that of the C rats. There was now a significant insulin response to glucose at 30 min ($P < .001$), but it was smaller than in the C rats and resulted in a lower insulin/glucose ratio (0.19 ± 0.009 ng/mmol, n = 39; $P < .001$ vs. 12-wk-old C rats). The serum insulin levels of the LP rats remained lower than those of the C rats throughout the test.

Tissue composition. The cell size of the C rats, as evidenced by the tissue protein/DNA ratio, increased with age in all the tissues studied ($P < .001$; Table 2). The ratios were

TABLE 2
Protein/DNA ratios in various tissues of rats fed diets with different protein content between 3 and 6 wk of age

Age (wk)	Diet	n	Small intestine	Cardiac muscle	Lung	Liver	Kidney	Skeletal muscle
3		24	36 ± 3	29 ± 2	13 ± 1	48 ± 2	31 ± 2	79 ± 4
6	LP	24	31 ± 2*	34 ± 2*	19 ± 1*	29 ± 1*	37 ± 3*	97 ± 6*
6	C	29	48 ± 2	56 ± 2	30 ± 2	50 ± 3	61 ± 3	138 ± 4
12	LP	30	64 ± 3	64 ± 2*	22 ± 1*	48 ± 2*	63 ± 4	145 ± 7*
12	C	36	58 ± 2	99 ± 3	33 ± 1	75 ± 4	70 ± 3	265 ± 9

Wistar rats were weaned at 3 wk of age onto diets containing 5% (LP) or 15% (C) protein and maintained ad libitum on respective diets for 3 wk. At 6 wk of age they were returned to commercial rat chow (protein content 18.1%) for the remainder of the experiment. Tissue samples were obtained from 3-, 6-, and 12-wk-old rats. Protein and DNA contents were assayed in homogenates of the samples, and protein/DNA ratio was calculated. Values are given as means ± SE for the number of observations shown.
*Significance of difference between animals of the same age but on different diets: $P < .001$.

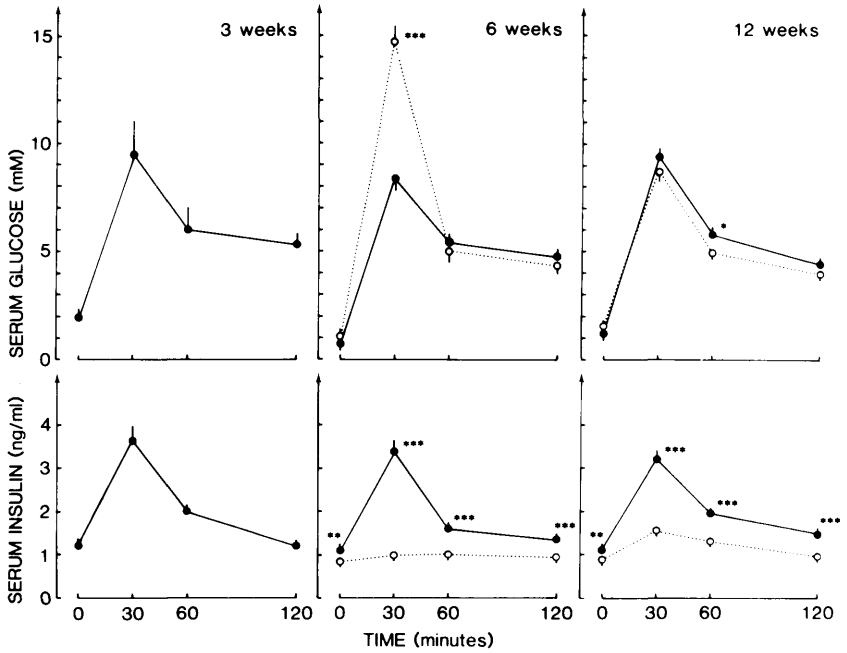

FIG. 2. Glucose tolerance and insulin secretory response to glucose in 3-, 6-, and 12-wk-old rats. Rats were weaned at 3 wk of age onto control diet (15% protein; ●) or low-protein diet (5% protein; ○) and maintained ad libitum on respective diets up to 6 wk of age, when they were returned to commercial rat chow (protein content 18.1%) for remainder of experiment. Serum glucose and insulin values are means ± SE for 25–40 rats. Significance of difference between rats on different dietary protocols: *$P < .05$, **$P < .01$, and ***$P < .001$.

similar in males and females; the results were pooled. In 6-wk-old LP rats the protein/DNA ratio of the liver had decreased, and in the other tissues the increase observed from 3 to 6 wk in C rats had been inhibited. The ratios of all the tissues of 6-wk-old LP rats were lower than those of C rats of the same age. From 6 to 12 wk of age the ratios increased in all the tissues of the LP rats, but in cardiac muscle, lung, liver, and skeletal muscle the ratios of the 12-wk-old LP rats remained lower than those of the 12-wk-old C rats.

DISCUSSION

This study confirms previous observations of an almost complete cessation of growth of young rats weaned onto a diet low in protein (<6%) (22,29–33). In this context, note that rats fed such diets reduce their food intake to a level just sufficient to maintain body weight. The state induced is therefore one of protein-calorie malnutrition and not a pure protein deficiency (30,34,35).

The impaired glucose tolerance and absent insulin secretory response to glucose confirm previous studies of protein-calorie–malnourished rats (22–24). However, the incomplete recovery of the insulin secretory response suggests permanent damage and/or functional impairment of the pancreatic β-cell. The atrophy and disruption of normal pancreatic morphology observed in both human kwashiorkor (36) and experimental protein-calorie malnutrition (37–39) could possibly reduce the number of islets. However, morphological studies have suggested that the islets of Langerhans are less affected than the exocrine parenchyma (37,38). Moreover, the ability of the β-cells to recover some of their insulin secretory response would suggest a functional impairment rather than an irreversible destruction. During experimental protein-calorie malnutrition, both the total islet mass (32) and the size of individual β-cells (38) are reduced, and it is possible that the endocrine pancreas does not recover the cell size or attain the normal cell number required to maintain an adequate insulin secretory response to glucose.

Note that, although the glucose tolerance was severely impaired in 6-wk-old LP rats, the glucose levels rapidly returned to normal during the GTT, despite the absent insulin secretory response. This observation would suggest an increased peripheral glucose utilization and sensitivity to insulin. An increased hypoglycemic response to exogenous insulin has indeed been reported in protein-calorie–malnourished rats (22). Our findings could therefore indicate that the impairment of glucose tolerance during protein-calorie malnutrition is caused by deficient insulin secretion rather than by changes in peripheral insulin action. An increased peripheral glucose utilization and sensitivity to insulin is also suggested in the 12-wk-old LP rats, which have normal glucose tolerance despite a blunted insulin secretory response to glucose. It is nevertheless conceivable that these animals could have a diminished capacity to respond to diabetogenic stimuli with an increased insulin secretion. Protein-calorie malnutrition early in life could thus predispose an impaired glucose tolerance and/or diabetes later in life, even though the acute effects of malnutrition have been reversed with an adequate diet.

With treatment of protein-calorie malnutrition, cell replication and longitudinal growth is rapidly resumed at normal or even increased rates. Thus, the observed persisting impairment of insulin secretion and the similar finding in treated human protein-calorie deficiency (9–11,14,16,40) do not seem to affect growth during or after recovery from malnutrition. However, the persisting reduction of the protein/DNA ratio in several tissues, which has also been observed in humans (35), indicates that the cellular protein accretion and growth in size is impaired, despite an adequate diet. This finding may be related to decreased insulin secretion because this hormone stimulates protein biosynthesis and cytoplasmic growth rather than cell replication (41,42). Early

malnutrition thus causes long-lasting alterations in tissue and organ composition, but whether function is affected or a predisposition to future diseases is incurred remains uncertain.

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REFERENCES

1. WHO Study Group: *Diabetes Mellitus*. Geneva, World Health Org., 1985, p. 20–25 (Tech. Rep. Ser. 727)
2. West KM: Special types of diabetes. Diseases of the exocrine pancreas. In *Epidemiology of Diabetes and Its Vascular Lesions*. New York, Elsevier, 1978, p. 324–34
3. Keen H, Ekoe JM: The geography of diabetes mellitus. *Br Med Bull* 40:359–65, 1984
4. Rao RH: The role of undernutrition in the pathogenesis of diabetes mellitus. *Diabetes Care* 7:595–601, 1984
5. Slone D, Taitz LS, Gilchrist GS: Aspects of carbohydrate metabolism in kwashiorkor with special reference to spontaneous hypoglycemia. *Br Med J* 1:32–34, 1961
6. Bowie MD: Intravenous glucose tolerance in kwashiorkor and marasmus. *S Afr Med J* 38:328–29, 1964
7. Baig HA, Edozien JC: Carbohydrate metabolism in kwashiorkor. *Lancet* 2:662–65, 1965
8. Hadden DR: Glucose, free fatty acid, and insulin interrelations in kwashiorkor and marasmus. *Lancet* 2:589–93, 1967
9. James WPT, Coore HG: Persistent impairment of insulin secretion and glucose tolerance after malnutrition. *Am J Clin Nutr* 23:386–89, 1970
10. Becker DJ, Pimstone BL, Hansen JDL, Hendricks S: Insulin secretion in protein-calorie malnutrition. I. Quantitative abnormalities and response to treatment. *Diabetes* 20:542–51, 1971
11. Milner RDG: Metabolic and hormonal responses to glucose and glucagon in patients with infantile malnutrition. *Pediatr Res* 5:33–39, 1971
12. Godard C, Zahnd GR: Growth hormone and insulin in severe infantile malnutrition. II. Plasma insulin and growth hormone during intravenous glucose tolerance test. *Helv Paediatr Acta* 26:276–85, 1971
13. Becker DJ, Pimstone BL, Hansen JDL, MacHutchon B, Drysdale D: Patterns of insulin response to glucose in protein-calorie malnutrition. *Am J Clin Nutr* 25:499–505, 1972
14. Milner RDG: Insulin secretion in human protein-calorie deficiency. *Proc Nutr Soc* 31:219–23, 1972
15. Rao KSJ, Raghuramulu N: Insulin secretion in kwashiorkor. *J Clin Endocrinol Metab* 35:63–66, 1972
16. Becker DJ, Pimstone BL, Hansen JDL: The relation between insulin secretion, glucose tolerance, growth hormone, and serum proteins in protein-calorie malnutrition. *Pediatr Res* 9:35–39, 1975
17. Cook GC: Glucose tolerance after kwashiorkor. *Nature* (Lond) 215:1295–96, 1967
18. Cook GC: Glucose and starch tolerance after recovery from kwashiorkor. *Metabolism* 17:1073–83, 1968
19. Kajubi SK: The endocrine pancreas after kwashiorkor. *Am J Clin Nutr* 25:1140–42, 1972
20. Heard CRC: Effects of severe protein-calorie deficiency on the endocrine control of carbohydrate metabolism. *Diabetes* 15:78–89, 1966
21. Heard CRC, Turner MR: Glucose tolerance and related factors in dogs fed diets of suboptimal protein value. *Diabetes* 16:96–107, 1967
22. Weinkove C, Weinkove EA, Pimstone BL: Glucose tolerance and insulin release in malnourished rats. *Clin Sci Mol Med* 50:153–63, 1976
23. Younoszai R, Dixit PK: Decreased insulin secretion by isolated pancreatic islets from rats fed 4% protein diet. *Proc Soc Exp Biol Med* 164:317–21, 1980
24. Levine LS, Wright PG, Marcus F: Failure to secrete immunoreactive insulin by rats fed a low protein diet. *Acta Endocrinol* 102:240–45, 1983
25. Syme G: The effect of protein-deficient isoenergetic diets on the growth of rat jejunal mucosa. *Br J Nutr* 48:25–36, 1982
26. Bradford MM: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72:248–54, 1976
27. Kissane JM, Robins E: The fluorometric measurement of deoxyribonucleic acid in animal tissues with special reference to the central nervous system. *J Biol Chem* 233:184–88, 1958
28. Hinegardner RT: An improved fluorometric assay for DNA. *Anal Biochem* 39:197–201, 1971
29. Widdowson EM, McCance RA: Effect of a low-protein diet on the chemical composition of the bodies and tissues of young rats. *Br J Nutr* 11:198–206, 1957
30. Hill DE, Holt AB, Parra A, Cheek DB: The influence of protein-calorie versus calorie restriction on the body composition and cellular growth of muscle and liver in weanling rats. *Johns Hopkins Med J* 127:146–63, 1970
31. Stead RH, Brock JF: Experimental protein-calorie malnutrition: rapid induction of protein depletion signs in early-weaned rats. *J Nutr* 102:1357–66, 1972
32. Weinkove C, Weinkove E, Timme A, Pimstone B: Pancreatic islets in malnourished rats. Quantitative histologic and electron microscopic findings. *Arch Pathol Lab Med* 101:266–69, 1977
33. Timson BF, Dudenhoeffer GA: The effect of severe dietary protein restriction on skeletal muscle fiber number, area and composition in weanling rats. *J Anim Sci* 61:416–22, 1985
34. Cabak V, Dickerson JWT, Widdowson EM: Response of young rats to deprivation of protein or of calories. *Br J Nutr* 17:601–16, 1963
35. Cheek DB, Hill DE, Cordano A, Graham GG: Malnutrition in infancy: changes in muscle and adipose tissue before and after rehabilitation. *Pediatr Res* 4:135–44, 1970
36. Trowell HC, Davies JNP, Dean RFA: *Kwashiorkor*. London, Arnold, 1954, p. 122–60
37. Volk BW, Lazarus SS: Rabbit pancreas in protein malnutrition (experimental kwashiorkor) and after cortisone administration. *Am J Pathol* 37:121–35, 1960
38. Platt BS, Stewart RJC: Experimental protein-calorie deficiency: histopathological changes in the endocrine glands of pigs. *J Endocrinol* 38:121–43, 1967
39. Madi K, Jervis HR, Anderson PR, Zimmerman MR: A protein-deficient diet. Effect on liver, pancreas, stomach, and small intestine of the rat. *Arch Pathol* 89:38–52, 1970
40. Graham GG, Cordano A, Blizzard RM, Cheek DB: Infantile malnutrition: changes in body composition during rehabilitation. *Pediatr Res* 3:579–89, 1969
41. Cheek DB, Hill DE: Muscle and liver cell growth: role of hormones and nutritional factors. *Fed Proc* 29:1503–509, 1970
42. Graystone JE, Cheek DB: The effects of reduced caloric intake and increased insulin-induced caloric intake on the cell growth of muscle, liver, and cerebrum and on skeletal collagen in the postweanling rat. *Pediatr Res* 3:66–76, 1969