Insulin Secretion in Relation to Adipose Tissue in Men

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SUMMARY

In a group of eighty-one fifty-five-year-old men, randomly selected from the total population, and in a group of twelve medical students, twenty-three years of age, body composition and adipose tissue fat cell size and number were determined. A 100 gm. peroral glucose tolerance test was performed, and blood glucose and plasma immunoreactive insulin were examined.

The older men showed higher glucose and particularly higher insulin responses after the glucose tolerance test than the young men. In lean older men, selected after ponderal index or body fat measurements, this was less pronounced. When a group of older men with the same fat cell diameter as the younger men was selected, the differences in glucose-insulin responses were still smaller and almost nonexisting between the two age groups. Fat cell diameter correlated only partially with ponderal index and body fat.

It was concluded that adipose tissue fat cell size is a more important determinant than adipose tissue size for the glucose and insulin response to a glucose tolerance test. Age has a relatively minor influence. DIABETES *20*:65-70, February, 1971.

Obesity is one of several clinical entities presumed to be characterized by increased levels of plasma insulin.[1-3] Since age and sex are factors which may influence insulin secretion and thereby complicate comparisons between patient and control groups, it is essential that these factors be strictly controlled if pertinent comparisons are to be made. In some studies,[4-7] age has been found to influence glucose tolerance and insulin secretion, while in others no correlations were found between age and glucose tolerance or insulin secretion.[8,9] Insulin secretion is also influenced by sex,[4,5,10-12] although fasting insulin has not been found different between sexes.[13]

Obesity is associated with an increased insulin secretion both in terms of an increased absolute amount of insulin in plasma,[1] and an increased insulin secretion in relation to transported glucose.[2,3] A close association between insulin secretion and the size of adipose tissue is suggested by several investigations.[14-17] Salans et al.[17] found that when six obese patients with expanded adipose tissue fat cells were reduced in weight, fat cell size and insulin secretion after a glucose tolerance test decreased. Since the insulin response in vitro of glucose oxidation to carbon dioxide was found to be inversely correlated to fat cell size, it was suggested that fat cell size and insulin secretion in vivo were in some way related.

In order to elucidate this question further, the correlations between glucose tolerance, insulin secretion, and different fat tissue factors were analyzed in a population of randomly selected fifty-five-year-old men. In order to analyze the influence of age when adipose tissue factors had been taken into consideration, another group of young men were studied for comparisons.

In the present study, large adipose tissue fat cell diameter, whether connected with obesity or not, was a significant determinant of high insulin output. The fat cell diameter appeared to be more closely related to insulin secretion in young and middle-aged men than did ponderal index or body fat.

A preliminary report of the present work was presented elsewhere.[18]

MATERIALS AND METHODS

The city of Göteborg, Sweden, is situated on the west coast and has approximately 400,000 inhabitants. The Revenue Office of the city must by law keep an up-to-date record of all people living in the city. All men born in 1913 on a date which is an even multiple of three were selected for a previous investigation.[19] The attendance was 92 per cent. For the present investigation, all men born on the sixth of every month were investigated in 1968. Of the eighty-five patients called, eighty-one volunteered for the study.

Medical students, twelve men of twenty-two to twenty-

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four years of age (average twenty-three years) were studied similarly.

After an overnight fast the subjects reported to the laboratory in the morning. Physical activity was avoided and smoking was not allowed. Heparinized venous blood was utilized for determination of blood glucose[20] and plasma immunoreactive insulin[21] before and 30, 60, 90, and 120 minutes after the ingestion of 100 gm. of glucose in 200 ml. water.

Body weight, height, waist circumference, and skin fold thickness in the triceps, subscapular, and abdominal regions were measured.[19] Body fat was estimated according to Moore et al.[22] from measurements of total body water and exchangeable potassium with isotope dilution methods[23,24] in another subsample of the original population of the men born in 1913 (to be published). The relations between body fat (BF) and the average of skinfold thicknesses (SF) and waist circumference (WC) were estimated in a computer. The regression equation $BF = 0.381 \times WC + 0.019 \times SF - 24.783$ yielded the highest correlation coefficient (0.87). Body fat was then calculated from this equation in the present subsample of the men born in 1913 by utilizing waist circumference and skinfold measurements. In the young men, body composition was determined directly.[22-24]

In forty-nine of the older men and in eleven young men, fat cell diameter was determined in adipose tissue specimens obtained by a percutaneous needle biopsy method described by Hirsch et al.[25] These specimens were quickly fixed in formalin, freeze-cut to appropriate slice thickness (about 200 μ). Fat cells were then measured with an ocular micrometer in the microscope.[26] With a 2 mm. diameter needle, a few shreds of adipose tissue are aspirated from the subcutaneous fat depot. This tissue is then kept in physiological saline for a short time before being fixed for ten minutes in 30 per cent formalin. This fixation causes no deformation and apparently no breakage of fat cells. This fixing makes it possible to make 200 μ slices from the tissue shreds, which have been frozen in carbon dioxide snow. These slices are transferred immediately to a small cup of physiological saline and covered with a siliconized thin glass plate. This avoids drying and deformation of the cells. After randomization procedures, cells are measured with an ocular micrometer and a normal distribution of cell sizes is obtained:

The method has been compared with the osmium tetraoxide method of Hirsch and Gallian[27] and found to agree. Total fat cell number was estimated by dividing total body fat with an average fat cell weight calculated according to Hirsch and Gallian.[27]

Measurements of fat cell diameter in the gluteal region and abdominal region to the left and below the umbilicus were performed in the older men and were not different. The abdominal fat cell diameter was therefore utilized for calculations throughout.

RESULTS

In figures 1 and 2 are shown the glucose and insulin values after the glucose tolerance test in both groups of men. The older men had higher glucose values ($p < 0.005$) at sixty and ninety minutes. The difference between the groups was more pronounced for insulin, and significant ($p < 0.05$ or lower) at all times except the fasting value (figure 2). The sum of all glucose values during the glucose tolerance test was

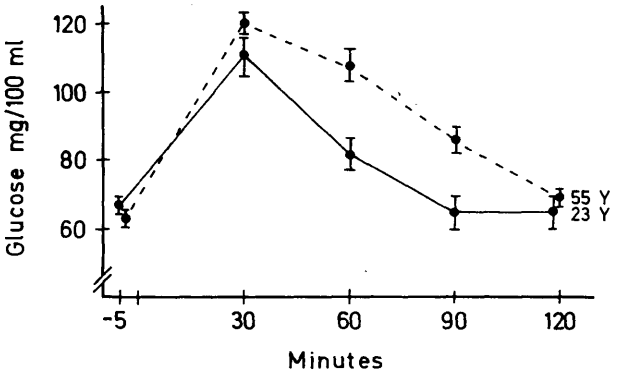

FIG. 1. Blood glucose values in fifty-five and twenty-three-year-old men after a 100 gm. peroral glucose tolerance test. Means ± SEM.

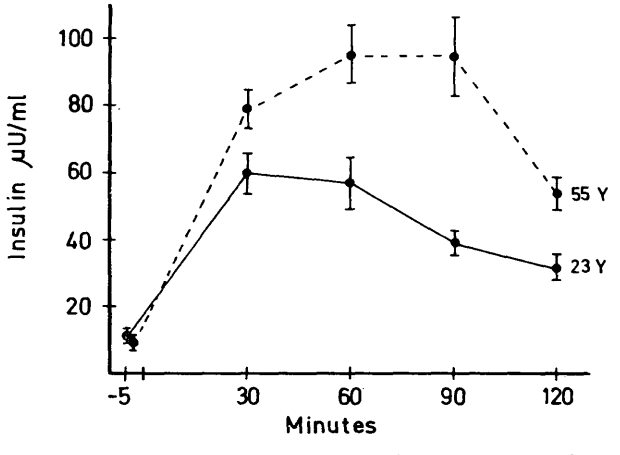

FIG. 2. Plasma insulin values in fifty-five and twenty-three-year-old men after a 100 gm. peroral glucose tolerance test. Means ± SEM.

TABLE 1

Body fat of young and middle-aged men divided in ponderal index (height/weight, cm./kg.) subgroups. Mean ± standard deviation.

	23-year old men	55-year-old men			
		All men (100%)	Lightest men (20%)	Middle weight (60%)	Heaviest men (20%)
Ponderal index	2.72±0.27	2.36±0.30	2.83±0.20	2.32±0.12	2.01±0.57
Body fat (kg.)	10.0 ±3.7	16.3 ±5.6	9.4 ±3.3	15.8 ±3.7	22.8 ±3.5
Number of men	12	75	15	45	15

497 ± 15 and 391 ± 20 mg. per cent (mean ± SEM) for middle-aged and young men respectively ($p < 0.005$); corresponding insulin values were 320 ± 30 and 200 ± 21 μU./ml. ($p < 0.001$).

The differences observed between the two age groups of men were examined in relation to the influence of different obesity factors. Therefore, the older men were first divided with a simple ponderal index (height/weight, cm./kg.) into subgroups of a high and low quintile and a remaining middle portion. The ponderal index and body fat estimation of the older men indicated that they were heavier than the young men in total and in all subgroups ($p < 0.001$) except for the lightest group where no difference was found in comparison with the young men (table 1).

When the glucose and insulin responses of the older and younger men were compared, it was found that the two heavier groups of older men had significantly higher values than had the young men on several time points. Glucose was higher in middle-weight older men (figure 3). Insulin was higher in middle-weight older men at 90 and 120 minutes ($p < 0.02$ or lower), and higher in heavy-weight older men at 30, 60, 90, and 120 minutes ($p < 0.05$ or lower) (figure 4). The leanest quintile of older men had higher glucose values than the young men at sixty and ninety minutes ($p < 0.01$) (figure 3), and higher insulin concentrations at ninety minutes ($p < 0.05$) (figure 4).

The number of older men with determination of fat cell diameter was too small to allow a subdivision into quintiles in accordance with the procedure for ponderal index and body fat. The findings of the older men were therefore divided into two parts separated by the median fat cell diameter. The average fat cell diameter of the older men (102 ± 16 μ) was significantly

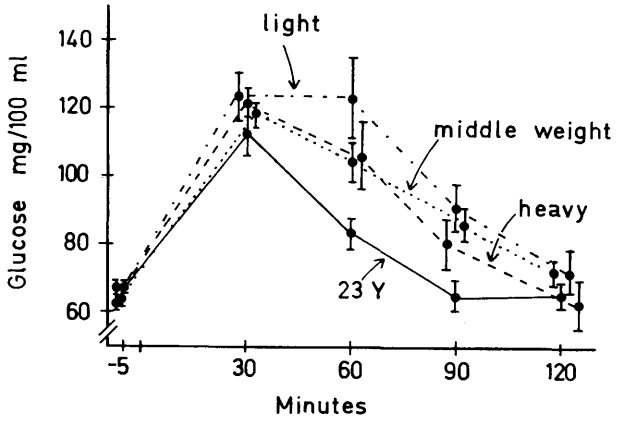

FIG. 3. Blood glucose values in middle-aged men, divided into ponderal index subgroups, and in young men after a 100 gm. peroral glucose tolerance test. Means ± SEM. Lightest 20 per cent of middle-aged men = pointed-dashed line. Middle 60 per cent of middle-aged men = pointed line. Heaviest 20 per cent of middle-aged men = dashed line. Young men = whole line.

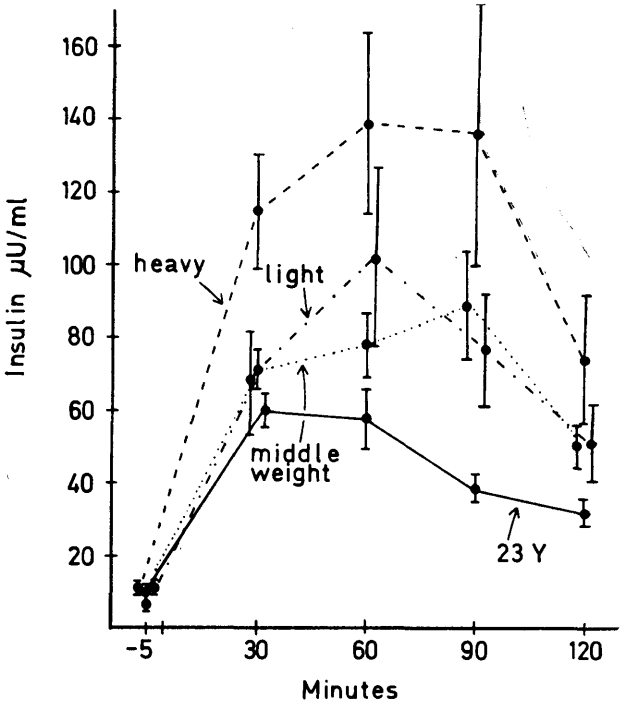

FIG. 4. Plasma insulin values in middle-aged men, divided into ponderal index subgroups, and in young men after a 100 gm. peroral glucose tolerance test. Means ± SEM. Abbreviations and symbols as in figure 3.

TABLE 2

Ponderal index (height/weight, cm./kg.) and body fat of young and middle-aged men divided in two halves after their adipose tissue fat cell diameter. Mean ± standard deviation.

	23-year old men	55-year-old men	
		The half of the material with smallest fat cells	The half of the material with largest fat cells
Fat cell diameter (u.)	94±7	87±14	115±9
Ponderal index	2.69±0.27	2.41± 0.28	2.33±0.27
Body fat (kg.)	10.0 ±3.7	14.3 ± 5.6	16.9 ±4.7
Number of men	11	23	22

higher than that of the young men (94 ± 7) ($p < 0.001$). The half of the older men with smaller fat cells had a mean fat cell diameter which was not different from that of the young men, while the other half had a significantly larger mean fat cell ($p < 0.001$) (table 2).

When the variables for measurement of obesity and fat cell size were compared, it was found that older men with large or small fat cells had similar ponderal indices and body fat. The young men, however, were lighter (higher ponderal index) and had less body fat than both the older groups ($p < 0.01$ or lower) (table 2). In older men the ponderal index was not correlated with fat cell diameter while body fat was ($r = 0.44$, $p < 0.005$).

The glucose and insulin values of the groups divided according to fat cell diameter are seen in figures 5 and 6. Older men with large fat cells had higher glucose values at sixty and ninety minutes ($p < 0.02$), and higher insulin values at 60, 90, and 120 minutes ($p < 0.02$ or lower) than did the young men. Also, the older men with fat cells of the same size as the young men had a higher glucose value only at ninety minutes ($p < 0.05$), while the insulin values were not different.

Within the group of middle-aged men, fasting insulin and sum of the insulin values during glucose tolerance test were correlated positively with fat cell diameter but negatively with fat cell number (table 3).

DISCUSSION

The middle-aged men tested in the present study had higher insulin and glucose responses after a peroral 100

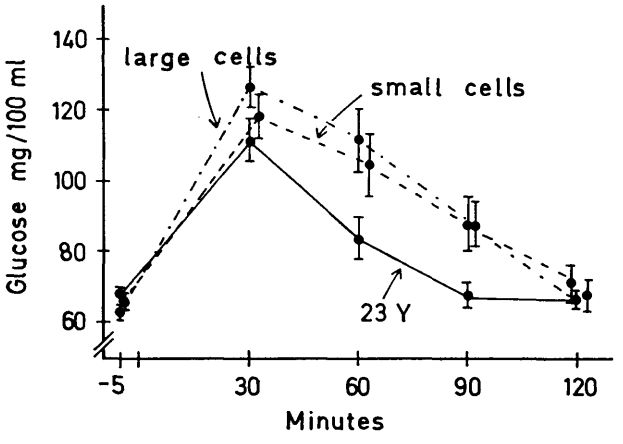

FIG. 5. Blood glucose values in middle-aged men, divided in two halves according to adipose tissue cell diameter, and in young men after a 100 gm. peroral glucose tolerance test. Means ± SEM. Small fat cells = dashed line. Large fat cells = pointed-dashed line. Young men = whole line.

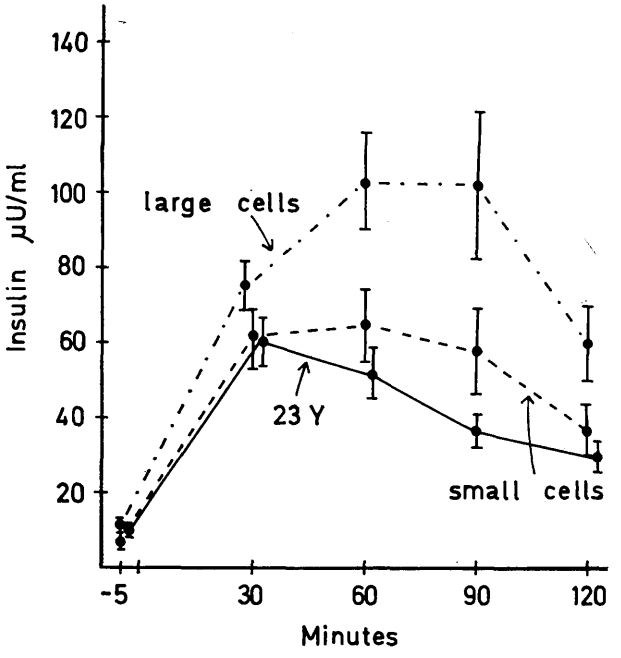

FIG. 6. Plasma insulin values in middle-aged men, divided in two halves according to adipose tissue cell diameter, and in young men after a 100 gm. peroral glucose tolerance test. Means ± SEM. Abbreviations and symbols as in figure 5.

TABLE 3

Linear regression equations between insulin and glucose values during glucose tolerance test, and adipose tissue fat cell diameter or number in middle-aged men.

x	Mean ± SD	y	Mean ± SD	Regression equation	Correlation coefficient	p
Fasting insulin	10±5 μU./ml.	Fat cell diameter	102±16	y=1.61x +86	0.51	<0.01
Sum of insulin during load	320±232 μU./ml.	Fat cell diameter	102±16	y=0.04x +91	0.37	<0.00
Fasting insulin	10±5 μU./ml.	Fat cell number (10^{10})	3.3± 2.0	y=0.13x + 4.5	—0.38	<0.02
Sum of insulin during load	320±232 μU./ml.	Fat cell number (10^{10})	3.3± 2.0	y=0.002x+ 3.9	—0.20	n.s.

gm. glucose tolerance test than did the young men. The increase was more pronounced for insulin than for glucose. This suggested that the difference might be explained at least in part by a higher amount of body fat in the older men, because body fat increases with age,[28] and obesity is accompanied by increased plasma insulin levels.[2,3] Therefore the influence of body fat on the increased glucose-insulin response in the older men was analyzed in different ways. The responses of older men with the same ponderal index or body fat as the young men were little different from those of the young men. When fat cell size was equalized in the young and older men, all differences in the glucose tolerance test disappeared except the glucose value at sixty minutes, which was slightly higher in the older group.

Thus, of the fat factors analyzed, fat cell diameter apparently had the greatest correlation with insulin secretion. It is noteworthy that fat cell size did not correlate with ponderal index and only partially with body fat. This implies that large fat cells can be found without obesity and vice versa. Furthermore, these findings suggest that the influence of obesity on glucose tolerance and insulin production is mediated by the size of adipose tissue fat cells rather than the amount of adipose tissue. In line with this concept is the absence of a positive correlation between any of the glucose and insulin values and the number of adipose tissue fat cells. The small remaining difference between the young and older men with the same fat cell size suggests that the influence of age on glucose tolerance and insulin production is rather limited.

It is not known how insulin production and enlarged fat cells might be causally related. Salans et al.[17] have suggested that there is a decreased insulin effect on glucose uptake in enlarged fat cells. It seems unlikely that this would cause a compensatory hyperinsulinemia, however, because total glucose uptake in adipose tissue is small when calculated from both in vitro data[29] and uptake of radioactive glucose in vivo.[30] Another possible explanation is that large fat cells produce an excess of fatty acids which then interfere with glucose uptake in the periphery,[31] causing increased compensatory secretion of insulin. This is unlikely in view of the fact that plasma glycerol, as a measure of adipose tissue lipid mobilization, did not correlate with fat cell size in the present material of middle-aged men, and correlated negatively with fasting insulin or sum of insulin during glucose tolerance test.[18] Finally, it may be considered that fat cell enlargement is secondary to an increased insulin production. This is suggested by the fact that when glucose is injected intravenously in obese patients, the plasma insulin concentration is higher, and more glucose is taken up in adipose tissue triglycerides of enlarged fat cells than in controls.[30]

Enlargement of fat cells is caused by a positive caloric balance.[32] The results presented here suggest that the increase in insulin production with age is largely due to a positive caloric balance, produced by an increased dietary intake and/or a decreased physical activity.

REFERENCES

[1] Karam, J. H., Grodsky, G. M., and Forsham, P. H.: Excessive insulin response to glucose in obese subjects as measured by immunochemical method. Diabetes *12*:197-204, 1963.

[2] Perley, M., and Kipnis, D. M.: Plasma insulin responses to glucose and tolbutamide of normal weight and obese diabetic and nondiabetic subjects. Diabetes *15*:867-74, 1966.

[3] Luft, R., Cerasi, E., and Anderson, B.: Obesity as an additional factor in the pathogenesis of diabetes. Acta Endocr. (Kobenhavn) *59*:344-52, 1968.

[4] Hayner, N. S., Kjelsberg, M. O., Epstein, F. H., and Francis, Th.: Carbohydrate tolerance and diabetes in a total community, Tecumseh, Michigan. Diabetes *14*:413-23, 1965.

[5] Marine, N., Vinik, A. I., Edelstein, I., and Jackson, W. P. U.: Diabetes, hyperglycemia and glucosuria among Indians, Malays and Africans (Bantu) in Cape Town (South

Africa). Diabetes *18*:840-57, 1969.

[6] Chlouverakis, C., Jarrett, R. J., and Keen, H.: Glucose tolerance, age and circulating insulin. Lancet *1*:806-09, 1967.

[7] Margio, S.: The insulin secretion in old age. Acta Diabet. Lat. 5 (suppl. 1):359-69, 1968.

[8] Schilling, W. H., Oberdisse, K., Huter, K. A., and Blank, H.: Vergleichende Untersuchung mit der oralen und intravenösen Glucosebelastung zur Erfassung einer verminderten Kohlenhydrattoleranz. Diabetologia *1*:187-94, 1965.

[9] Welborn, T. A., Rubenstein, A. H., Haslam, R., and Fraser, R.: Normal insulin response to glucose. Lancet *1*: 280-84, 1966.

[10] Rubenstein, A. H., Seftel, H. C., Miller, K., Bersohn, I., and Wright, A. D.: Metabolic response to oral glucose in healthy South African white, Indian and African subjects. Brit. Med. J. *1*:748-51, 1969.

[11] Boyns, D. R., Crossley, J. N., Abrams, M. E., Jarrett, R. J., and Keen, H.: Oral glucose tolerance and related factors in a normal population sample—I. Blood sugar, plasma insulin, glyceride, and cholesterol measurements and the effects of age and sex. Brit. Med. J. *1*:595-98, 1969.

[12] Abrams, M. E., Jarrett, R. J., Keen, H., Boyns, D. R., and Crossley, J. N.: Oral glucose tolerance and related factors in a normal population sample—II. Interrelationship of glycerides, cholesterol, and other factors with the glucose and insulin response. Brit. Med. J. *1*:599-602, 1969.

[13] Hagerup, L., Deckert, T., and Larsen, M.: Fasting insulin in a normal population (In Danish). Nord. Med. *84*:198-201, 1970.

[14] Karam, J. H., Grodsky, G. M., Pavlatos, F. C., and Forsham, P. H.: Critical factors in excessive serum-insulin response to glucose. Lancet *1*:286-89, 1965.

[15] Liebermeister, H., Daweke, H., Gries, F. A., Schilling, W. H., Gruneklee, D., Probst, G., and Jahnke, K.: Einfluss des Gewichtsreduktion auf Metabolite des Kohlenhydrat—und Fettstoffwechsels und auf das Verhalten des Seruminsulins bei Adipositas. Diabetologia *4*:123-32, 168.

[16] Bagdade, J. D., Bierman, E. L., and Porte, D., Jr.: The significance of basal insulin levels in the evaluation of the insulin response to glucose in diabetic and nondiabetic subjects. J. Clin. Invest. *46*:1549-57, 1967.

[17] Salans, L. B., Knittle, J. L., and Hirsch, J.: The role of adipose cell size and adipose tissue insulin sensitivity in the carbohydrate intolerance of human obesity. J. Clin. Invest. *47*:153-65, 1968.

[18] Björntorp, P., Gustafson, A., and Tibblin, G.: Relationships between adipose tissue cellularity and carbohydrate and lipid metabolism in a randomly selected population. Proc. of the Second Int. Symp. on Atherosclerosis, Berlin, Springer, 1970, p. 374.

[19] Tibblin, G.: High blood pressure in men aged 50. Acta Med. Scand. suppl. 470, 1967.

[20] Levin, K., and Linde, S.: Determination of glucose in blood, cerebrospinal fluid and urine with a new glucose-oxidase reagent. J. Swed. Med. Assoc. 59:3016-36, 1962.

[21] Hales, C. N., and Randle, P. J.: Immunoassay of insulin with insulin antibody precipitate. Lancet *1*:200, 1963.

[22] Moore, F. D., Olesen, K. H., McMurray, J. D., Parker, H. V., Ball, M.R., and Boyden, C. M.: The body cell and its supporting environment. Philadelphia, Saunders, 1963.

[23] Lindholm, B.: Body cell mass during long-term cortisone treatment in asthmatic subjects. Acta Endocr. (Kobenhavn) 55:202-21, 1967.

[24] Lindholm, B.: Body cell mass during long-term treatment with cortisone and anabolic steroids in asthmatic subjects. Acta Endocr. (Kobenhavn) 55:222-39, 1967.

[25] Hirsch, J., Farquhar, J. W., Ahrens, E. H., Jr., Peterson, M. L., and Stoffel, W.: Studies of adipose tissue in man. A microtechnic for sampling and analysis. Amer. J. Clin. Nutr. *8*:499-511, 1960.

[26] Björntorp, P., Sjöström, L., and Vrana, J.: In preparation, 1970.

[27] Hirsch, J., and Gallian, E.: Methods for the determination of adipose cell size in man and animals. J. Lipid Res. 9:110-19, 1968.

[28] Keys, A., and Brozek, J.: Body fat in adult man. Physiol. Rev. 33:245-325, 1953.

29 Björntorp, P., Scherstén, T., and Gottfries, A.: Effects of glucose infusions on adipose tissue lipogenesis in man. Acta Med. Scand. *183*:565-71, 1968.

[30] Björntorp, P., and Larsson, B.: To be published, 1970.

[31] Randle, P. J., Garland, P. B., Hales, C. N., and Newsholme, E. A.: The glucose fatty acid cycle. Its role in insulin sensitivity and the metabolic disturbance of diabetes mellitus. Lancet *1*:785-89, 1963.

[32] Sims, E., and Horton, E. S.: Endocrine and metabolic adaptation to obesity and starvation. Amer. J. Clin. Nutr. *21*:1455-70, 1968.