THE INFLUENCE OF PREMEDICATION, ANAESTHESIA, AGE AND WEIGHT ON GLUCOSE UPTAKE INTO HUMAN ISOLATED SKELETAL MUSCLE

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- 1 The effect of the anaesthetic procedures and of the sex, age and weight of each patient on glucose uptake and glycogen content of human skeletal muscle has been studied *in vitro* in the presence and absence of insulin.
- 2 Statistical analysis indicated that the relationships between age and both glucose uptake and the response to insulin were significant, older patients in general having higher uptakes.
- 3 The glucose uptake was highly correlated with the three obesity indices (ponderal index, body mass index and percentage of the ideal weight).
- 4 The anaesthetic agents had no significant effect on glucose uptake.
- 5 The choice of premedication appeared to have a small effect on the basal glucose uptake level, but as the choice of premedication was also age related and age itself was a significant factor, this effect may not be of importance.
- 6 It is concluded that the age and the degree of obesity of the patients ought to be taken into account when studying samples of human muscle.

Introduction

In recent years a number of papers have described studies on glucose metabolism carried out on preparations of human isolated skeletal muscle (Magyar, Lehoczy & Marton, 1965; Holm & Schersten, 1972; Frayn, Adnitt & Turner, 1973; Nuttall, Barbosa & Gannon, 1974; Suominen, Forsberg, Heikkinen & Österback, 1974), and it seems likely that similar preparations will be used increasingly in the future for both biochemical and pharmacological investigations. However, little attention has been paid to possible variations in the response due to factors such as the anaesthetic agents used and the sex, age and weight of the patient from whom the muscle sample has been obtained. Bennis & Smith (1973) have shown that after halothane anaesthesia the metabolism of human adipose tissue is altered, but none of the workers mentioned above have investigated the possible effects of the anaesthetic agents used on the *in vitro* response of the skeletal muscle. Similarly the question of different responses in muscle from obese patients has not been investigated. Holm & Schersten (1972) attempted to study the effect of age, but they divided their patients into two age groups only (20-30 years and 45-70 years), and although they demonstrated a significantly higher level of glycogen in the younger group, they could only show a trend to

higher incorporation rates for glucose into various metabolites in younger patients. In the present study we have investigated the possible effects of the age, weight, sex and anaesthetic agents used, on glucose uptake by and glycogen content of human isolated gluteus maximus and gluteus medius muscle, both in the presence and absence of insulin. In order to study the weight and the degree of obesity of our patients we have used three measurements of obesity: ponderal index (Livi, 1897), body mass index (Keys, Fidanza, Karvonen, Kimura & Taylor, 1972) and percentage of the ideal weight using the Metropolitan Life Insurance Tables (1959) and studied the relationships between them.

Methods

Reagents

Crystalline beef insulin, glucagon low (Batch No. BJ-4609, potency 24.0 U/mg) was from Eli Lilly & Co. Ltd, Indianapolis, U.S.A. Bovine serum albumin (Cohn fraction V) and glycogen (from shell fish) type II were from Sigma, London. Reagents for the glucose oxidase technique were from Hughes & Hughes (Enzymes) Ltd, Brentwood. All other reagents were of analytical grade.

Tissue and experimental design

Pieces of human skeletal muscle were obtained from gluteus maximus or gluteus medius muscle of patients undergoing surgery for total hip joint replacement. The patients had been fasted for 8-14 h before surgery. Two forms of intramuscular premedication were used, papaveretum (Omnopon) (10-20 mg) and scopolamine (0.2-0.4 mg) or pethidine (50-100 mg) and atropine (0.6 mg). Anaesthesia was induced intravenously with thiopentone sodium (5 mg/kg) and maintained with 66% nitrous oxide in oxygen, suxamethonium (50-100 mg) being used to facilitate oral intubation. In some patients trichloroethylene (Trilene), halothane or a combination of the two agents was used to increase the depth of anaesthesia. Neuromuscular blockade with either tubocurarine or pancuronium and intermittent positive pressure ventilation (IPPV) was used in 55% of the cases. The age range for the group of fourteen men and twenty-six women was 25-79 years (mean 60.4). For each patient the age, sex, weight and height and anaesthetic procedure used were recorded. The ponderal index (Seltzer, 1966), the height in inches divided by the cube root of the weight in pounds which ranged from 10.8 to 16.2 with a mean of 12.6; the body mass index (Keys *et al.*, 1972), the weight in g divided by the height squared in cm which ranged from 1.57 to 3.74 with a mean of 2.37 and the percentage of the ideal weight from the Metropolitan Life Insurance tables (1959) which ranged from -27% to +69.4% with a mean of +9.2%, were calculated for each patient.

Muscle samples from each patient were incubated either in the presence or absence of 100 μ U/ml of insulin. Two or more pieces of muscle were taken from each sample for each incubation whenever possible. The mean glucose uptake in the presence and absence of insulin was calculated for each patient. Also the glycogen content of the muscle at the end of the incubations was measured.

Preparation and incubation procedure

The incubation medium was Krebs-Henseleit bicarbonate buffer (Dawson, 1969), saturated in an ice bath with 95% oxygen and 5% carbon dioxide. After gassing, glucose and bovine serum albumin were added to a final concentration of 3 mg/ml and 2 mg/ml respectively. Albumin was used as Sönksen, Ellis, Lowy, Rutherford & Nabarro (1965) showed that this concentration prevents, to a large extent, the adsorption of insulin to glass. Insulin was added to the medium by dilution with the medium from a stock solution of 1 mg/ml in

0.03 M HCl to give a final concentration of 100 μ U/ml.

Tissue was collected in gassed ice-cold medium which contained no additions. Within 10 min after its removal, the tissue was dissected in the direction of the muscle fibres into pieces weighing 80-250 mg, after removal of any visible fat or fibrous tissue. As many pieces as possible were prepared from each sample. Each piece was gently blotted before being weighed on a torsion balance. Each muscle sample was then placed in a separate conical flask (25 ml) which contained 2 ml of the incubation medium with the appropriate additions. The flasks were flushed with 95% oxygen and 5% carbon dioxide, stoppered and incubated with shaking (60 strokes/min) at 37°C for 90 minutes. The tissue was then removed from the flask, blotted and dissolved in 5.4M KOH (2 ml) at 100°C prior to glycogen estimation. Control flasks (without muscle) were also incubated and the glucose concentration of these was also determined. The glucose uptake by the muscle was calculated from the decrease in the glucose concentration in the test compared with the control flasks and the results were expressed in mg glucose taken up g^{-1} wet weight of tissue in 90 min and the glycogen results as mg glycogen g^{-1} wet weight of tissue.

Glucose and glycogen estimations

Glucose was estimated by a glucose oxidase method using guaiacum as the colour reagent (Morley, Dawson & Marks, 1968). Glycogen was estimated by a method based on that of Walaas & Walaas (1950). After acid hydrolysis of the glycogen to glucose with 2N H_2SO_4 (1 ml), the glucose was estimated by the glucose oxidase-guaiacum method mentioned before.

Statistical treatment of the results

Scatter diagrams were drawn and Spearman's Rank correlation coefficients were computed to investigate the relationships between the obesity indices, the heights, the weights and the ages of the patients and the association between these variables and the glucose uptake levels with and without insulin. A nonparametric statistical technique was used as the distributions of the variables did not all appear to be normal.

Results

Table 1 shows the results for glucose uptake from one patient (female, 58 years) to indicate the degree of variation in uptake between different

Figure 1 Variation in glucose uptake into human isolated skeletal muscle with age. ● glucose uptake in the presence of insulin (100 μ U/ml); ○ glucose uptake in the absence of insulin.

muscle strips from the same patient and also to show that the weight of the muscle sample within the range studied had no effect on the level of glucose uptake when it was expressed in terms of mg glucose taken up g^{-1} wet weight of tissue in 90 minutes. We have also been able to confirm the reliability of the estimation, as we were able to obtain two muscle samples from another patient, 3 weeks apart when the other hip joint was replaced (male, 36 years, first occasion mean glucose uptake without insulin 2.1, repeat 2.1, with insulin 2.7 and repeat 2.8 mg glucose g^{-1} wet weight of

tissue in 90 min; first occasion glycogen content without insulin was 9.1 and when repeated was 8.8 and with insulin was 10.3 and then 9.7 mg of glycogen g^{-1} wet weight of tissue.

The Spearman's Rank correlation coefficients together with the corresponding levels of significance in Table 2 show the relationships between age, weight, height and the three obesity indices. From this table it can be seen that none of the obesity factors was correlated with height, but that all were strongly correlated with weight. In addition, the three indices are closely related to each other. Age did not appear to be related to height, weight or any of the obesity indices. The scatter diagram in Figure 1 shows that muscle samples from older patients tended to have higher levels of glucose uptake, both in the presence and absence of insulin. The Spearman's Rank correlation coefficients given in Table 3 show that this positive relationship between age and glucose uptake was highly significant. The correlations between glucose uptake with height, weight and the three obesity indices are also given in Table 3.

Scatter diagrams also showed that patients given atropine and pethidine, rather than papaveretum and scopolamine, were older on average and muscle from these patients tended to have higher glucose uptake levels. Similar scatter diagrams showed no significant association between the use of halothane and/or trichloroethylene and glucose uptake levels. Scatter diagrams showed a tendency for muscle samples from the more obese patients to give higher levels of muscle glycogen, this being significant when ponderal index was used as the measurement of obesity. No significant relationships were found between any of the other factors and the glycogen levels after incubation with or without insulin.

Table 1 Variation in glucose uptake in muscle strips from the same patient

Conditions	Weight of muscle strip (mg wet weight)	Glucose uptake (mg glucose taken up g^{-1} wet weight of tissue in 90 min)	Mean $\pm$ s.e. mean glucose uptake
No insulin	218	2.0	2.15 $\pm$ 0.17
	120	1.8	
	172	2.3	
	127	2.5	
With insulin (100 μ U/ml)	217	3.7	3.60 $\pm$ 0.15
	122	3.6	
	162	3.2	
	112	3.9	

Table 2 Spearman's rank correlation coefficients to show the relationships between age, weight, height and three obesity indices

<i>Variable</i>		<i>Correlation coefficient</i>	<i>Significance level of difference from zero</i>
Age	Height	-0.22	NS
Age	Weight	0.04	NS
Age	Ponderal index	-0.15	NS
Age	Body mass index	0.11	NS
Age	% ideal weight	0.10	NS
Height	Weight	0.69	0.1%
Height	Ponderal index	0.15	NS
Height	Body mass index	0.23	NS
Height	% ideal weight	0.11	NS
Weight	Ponderal index	-0.53	0.1%
Weight	Body mass index	0.83	0.1%
Weight	% ideal weight	0.74	0.1%
Ponderal index	Body mass index	-0.88	0.1%
Ponderal index	% ideal weight	-0.91	0.1%
Body mass index	% ideal weight	0.97	0.1%

Table 3 Spearman's rank correlation coefficients to show the relationships between age, weight, height, three obesity indices and glucose uptake

<i>Variable</i>		<i>Correlation coefficient</i>	<i>Significance level of difference from zero</i>
(A) Glucose uptake without insulin with			
Age		0.42	1%
Height		-0.45	1%
Weight		-0.01	NS
Ponderal index		-0.48	0.2%
Body mass index		0.36	5%
% ideal weight		0.40	1%
(B) Glucose uptake with insulin (100 μ U/ml)			
Age		0.57	0.1%
Height		-0.22	NS
Weight		0.18	NS
Ponderal index		-0.49	0.2%
Body mass index		0.45	1%
% ideal weight		0.46	1%

Discussion

There have been several investigations into the effect of halothane on carbohydrate metabolism both *in vitro* and *in vivo*. Mäkeläinen (1974) showed an elevation of blood glucose levels with halothane anaesthesia in patients undergoing minor elective surgery, and Bennis & Smith (1973), using human isolated fat cells, showed a decrease in levels of basal glycerol release and a less pronounced antilipolytic response to insulin, but were unable to detect changes in glucose incorporation with halothane. Reynolds (1974), using the rat isolated diaphragm preparation, studied the effect of halothane on phosphorylase activity of the muscle and was unable to demonstrate an effect on the basal activity of the enzyme, but did show a decrease in the response of the enzyme to noradrenaline. We have been unable to show any significant alteration in response to insulin or in the basal glucose uptake levels or glycogen content of the muscle when halothane had been used. However, the premedication seems to have had an effect on the basal glucose uptake level, but as the choice of premedication was found to be age related in our study and age was itself a significant factor in determining the level of glucose uptake, the effect of the premedication may not be of importance.

Before considering the effect of weight and obesity of the patients on the glucose uptake and glycogen content levels it is of importance to discuss the three obesity indices used and their interrelationships. Various indices of obesity obtained from the measurements of height and weight have been proposed since Livi in 1897 proposed his 'indice ponderali'. These indices have been reviewed by Khosla & Lowe (1967) who defined two criteria for any index, based on the fact that short people are no more likely to be obese than tall, so (1) the index must be independent of height (2) the index must be highly correlated with weight. In Table 2 it can be seen that none of the indices was significantly correlated with height, but all were highly correlated with weight and with each other. The correlation coefficient between weight and body mass index was largest and smallest for ponderal index. These findings agree with the work of Florey (1970) who found, using the two criteria of Khosla & Lowe (1967), that body mass index was the better ratio. Keys *et al.* (1972) also found that the ponderal index was the poorest of the obesity indices studied and body mass index slightly better than a simple ratio of weight to height. They also showed that ponderal index was the index least sensitive to changes in weight. The use of percentage ideal weight obtained from insurance tables as a

measure of obesity applied to the general population can be criticized in two ways: firstly the tables do not take age into consideration, referring to weight at age 25 years, and secondly the sample of people from whom the ideals are obtained are not a necessarily random sample from the population as it consists only of people wanting insurance. It is interesting to notice that in our sample we were unable to demonstrate a relationship between age and weight or degree of obesity. This probably reflects the fact that the more overweight patients were not selected for surgery.

It is of interest that although glucose uptake in the presence and absence of insulin was related to all three obesity indices, weight itself was not (Table 3). From this table it can also be seen that the levels of glucose uptake are positively correlated with the age of the patient. This is in contrast to the work of Holm & Schersten (1972) who although finding no significant correlation between glucose incorporation and age, did show a trend towards higher levels in younger patients and also a significant difference between the glycogen content in the two age groups, the younger group having higher levels. However, these authors considered patients in only two age groups (20-30 and 45-70 years), whereas we took age to be a continuous variable and also the majority of our patients was in the upper range. One factor which may account for the difference between their work and ours is that their muscle was obtained from four muscle sites (rectus abdominis, vastus lateralis, spina lica anterior superior and gastrocnemius) while we confined our sample to the gluteus region. Another factor is the physical activity of the patients used in each study. As all our patients were undergoing total hip joint replacement for an arthritic condition, their physical activity is likely to have been low. This probably does not apply to the patients studied by Holm & Schersten (1972), although they excluded athletes. They suggested that the higher glycogen content and the higher glucose uptake into the muscle seen in the younger patients may have been due to their being physically fitter than the older patients.

The increase in glucose uptake levels seen in the more obese is in agreement with the *in vivo* work of Rabinowitz & Zieler (1962) who used the forearm blood flow technique and showed that there was an increased glucose uptake in the obese compared with normal subjects. Whichelow & Butterfield (1971) on the other hand, using similar techniques, found a decrease in glucose uptake levels with increasing obesity. However, they were able to include some grossly obese patients in their study, their range of ponderal index, for example, being 8.78-14.32 compared with ours of 10.8-

16.2. This probably accounts for some of the differences in our findings.

In conclusion, these results suggest that because of the influence of age, obesity, and possibly premedication on glucose uptake, studies with human isolated skeletal muscle should be carried out on a within-subject basis wherever possible. When between-subject comparisons are to be made

patients should be matched for age, sex, obesity, premedication and anaesthetic procedure.

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