Inhibitory Effect of Caffeine on the In Vitro Uptake of Glucose by Rat Epididymal Adipose Tissue

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Summary. Using an experimental design in which the segments of adipose tissue were randomly distributed, the effect of caffeine on the uptake of glucose from a Krebs bicarbonate medium by isolated rat epididymal adipose tissue has been studied. It was found that caffeine significantly inhibited the uptake of glucose in this system. This observation is consistent with previous observations on the metabolism of glucose-1-¹⁴C, but does not differentiate between a primary effect on glucose uptake and an effect secondary to an inhibition of intermediary metabolism at some site within the cell.

Effet inhibiteur de la caféine sur la captation «in vitro» du glucose par le tissu adipeux épидидymaire du rat.

Résumé. On a étudié l'effet de la caféine sur la captation du glucose par le tissu adipeux épидидymaire du rat placé dans du liquide de Krebs bicarbonaté. On a utilisé pour cela un plan expérimental dans lequel les segments de tissu adipeux étaient distribués au hasard. On a trouvé que la caféine inhibait significativement la captation du glucose dans ce système. Cette observation est en accord avec les observations antérieures sur le métabolisme du glucose-1-C¹⁴, mais ne permet pas de différencier un effet

primaire sur la captation du glucose d'un effet secondaire à une inhibition du métabolisme intermédiaire en quelque site intracellulaire.

Hemmung der Glucose-Aufnahme des Ratten-Nebenhoden-Fettgewebes in vitro durch Coffein.

Zusammenfassung. Die Wirkung von Coffein auf die Glucoseaufnahme aus Krebs-Bicarbonat Puffer durch isoliertes Ratten-Nebenhoden-Fettgewebe wurde unter Anwendung eines gezielten Verteilungsschemas für die Fettgewebs-Segmente untersucht. Dabei ergab sich in diesem System eine signifikante Hemmung der Glucose-Aufnahme durch Coffein. Dieser Befund stimmt mit früheren Feststellungen über den Stoffwechsel von ¹⁴C-1-Glucose überein; es läßt sich jedoch nicht entscheiden, ob er durch eine primäre Wirkung auf die Glucose-Aufnahme bedingt ist oder ob er einen Sekundäreffekt nach Hemmung des Intermediärstoffwechsel an unbekannter Stelle innerhalb der Zelle darstellt.

Key-words: caffeine, glucose uptake, incubated adipose tissue, epididymal adipose tissue.

We have previously reported the main effects and interactions of caffeine and insulin on the metabolism of ¹⁴C labelled glucose by the isolated rat epididymal fat pad, finding that caffeine, 0.1 mg (0.5 μ mol) per ml, inhibited the basal and insulin-stimulated ¹⁴CO₂ production and ¹⁴C lipid incorporation from glucose-1-¹⁴C in Krebs bicarbonate buffer [1]. These results are compatible with diminished uptake of glucose by the fat pad, with impairment in the intracellular metabolism of glucose, or with both mechanisms operating simultaneously. The present studies were designed to demonstrate the specific effect of caffeine on glucose uptake by the isolated rat epididymal fat pad.

Materials and Methods

Six male Wistar rats (mean weight 230 gm) were housed in individual cages and allowed Purina Laboratory Chow and water *ad libitum*. Animals were killed by a blow to the head and the epididymal fat pads on each side were removed. From each pad, distal (D), medial (M), and proximal (P) segments (100-200 mg) were removed, weighed, and placed in 25 ml incubation flasks containing 7 ml of Krebs bicarbonate buffer at pH 7.4, with glucose 2.5 mg/ml (250 mg/100 ml). The 36 flasks were arranged in a completely random segment design. To 18 flasks was added 0.1 mg/ml of

caffeine¹, the other 18 serving as controls. Immediately prior to incubation, 2 ml of buffer was removed from each flask for estimation of glucose; the volume used for incubation was thus exactly 5 ml. The flasks were incubated with gentle agitation at 37° for three hours in a Dubnoff incubator. The Nelson-Somogyi method [4] was used to measure glucose content of the buffer removed from the flasks before and after incubation. To provide a series of blanks, two further sets of 12 flasks were incubated without adipose tissue, one set containing buffer, glucose, and caffeine, the other, buffer and glucose alone. Pre- and post-incubation glucose estimations were also performed on these flasks.

Results

In the incubation blank flasks without adipose tissue, there was a consistent increase in the concentration of glucose after incubation, averaging +8.12 mg per 100 ml in those containing caffeine, and +9.02 mg per 100 ml in those without. Thus the average increase over the three-hour incubation period for the 24 flasks was +8.57 mg per 100 ml, presumably reflecting a loss of 0.16 ml water during incubation, despite all precautions. We, therefore, calculated the amount of glucose present in all the post-incubation flasks which con-

¹ Caffeine Hydrate — Mann Research Laboratories.

tained adipose tissue, as based on the glucose concentration measured and an assumed volume of 4.84 ml of buffer rather than 5 ml. On this basis the differences between the pre- and post-incubation levels were expressed as uptake of glucose in μmols per gram of fat per three hours.

These figures are shown in ascending rank, together with the segments involved, in Table 1. The average uptake of glucose was $13.36 \mu\text{mol/g fat/3 hours}$ in the control flasks, and $5.47 \mu\text{mol/g fat/3 hours}$ in the flasks with caffeine — an average effect of caffeine of $-7.89 \mu\text{mol/g fat/3 hours}$. Scores were assigned to

Table 1. *Uptake of Glucose - $\mu\text{mol/g fat/3 hrs}$*

Caffeine			Control		
Segment	Uptake	Rank	Segment	Uptake	Rank
RP	24.661	35	RM	24.830	36
LP	21.357	31	RP	23.202	34
LM	17.039	27	LM	23.065	33
RM	16.340	26	PR	21.820	32
LP	15.864	24	LP	18.722	30
LM	9.328	18	LP	17.163	29
RD	4.938	16	RM	17.091	28
RD	4.635	15	LD	16.045	25
LD	4.227	14	LD	14.587	23
LD	3.085	12	RM	14.539	22
RP	2.578	11	LM	13.265	21
LD	2.518	10	RD	12.939	20
LP	0.000	7	LP	11.632	19
RP	-1.211	6	LM	9.028	17
RM	-2.172	5	LD	3.839	13
LM	-5.203	3	RD	1.452	9
RM	-6.171	2	RP	0.760	8
RD	-13.408	1	RD	3.497	4
Average 5.47			13.36		
Rank Sum. 263			Rank Sum. 403		

$P < .05$ two sided (Wilcoxon)

the individual figures according to rank, and the significance of the difference between treatments estimated using Wilcoxon's rank sums test [3] ($P < .05$ two sided). The more familiar t-test was not used because we were reluctant to make the necessary assumption of a normal distribution in the population; the samples

themselves were rather small and showed some troublesome deviation from normal. Therefore we preferred the Wilcoxon test, which does not depend on these assumptions, is much easier to calculate, and whose power-efficiency is 95.5%.

Discussion

The finding that caffeine significantly reduces the uptake of glucose by the isolated rat epididymal fat pad is not unexpected in view of the observations previously reported [1]. It also confirms the observations of Vaughan on the effects of caffeine on glucose uptake by adipose tissue [5]. Nevertheless, such data do not differentiate conclusively between a primary effect of caffeine on glucose uptake and a secondary effect which might be dependent upon a primary intra-cellular inhibitory action. Such an action would, by inhibiting the metabolism of glucose within the cell, be expected to produce a secondary inhibition of glucose uptake across the cell membrane. Further studies in progress using different substrates do in fact show an undoubted intracellular site of action, and strongly suggest a primary inhibition of lipid biosynthesis [2].

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