dietary caffeine, glucose tolerance and insulin sensitivity in mice.

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ABSTRACT

Mice consumed diets which contained caffeine and predominated in either corn starch, sucrose or hydrogenated vegetable oil. When compared by means of an oral glucose tolerance test to mice which had eaten the same diets without caffeine, it was found that they had a more rapid return of blood glucose to resting values. In vivo measurements of glucose uptake by diaphragm and epididymal fat pad revealed differences in this adaptation to caffeine. The adaptive mechanism appeared to vary with type of diet. When mice were given caffeine chronically in their drinking water there was evidence of adaptation occurring in the ability of muscle to increase glycogen stores in the presence of caffeine in vitro. No differences were found in other tissue glycogen levels.

INTRODUCTION

The role of the methyl xanthines, such as caffeine, in the regulation of cyclic 3',5'-adenosine monophosphate levels has led to an increased awareness of the importance of these dietary compounds in the control of the metabolic apparatus (Sutherland, 1972). Studies have previously reported direct effects on the pancreatic islets and peripheral changes (Kuftinac and Mayer, 1964; Mayer, 1966; Jankelson et al., 1967). With current evidence in the ultimate metabolic effects of the source of dietary calories, it was hypothesized that an interaction between diet and caffeine could be observed. It was impossible to decide a priori between the two effects of cyclic AMP-first its catabolic action on glycogen and lipid stores, and second its enhanced release of insulin from the pancreas. It was the purpose of this study to determine which of the known effects of the ingestion of caffeine (Jankelson et al., 1967; Wachman et al., 1970) would predominate.

MATERIALS & METHODS

Adult male albino mice (20-25g) of the Charles River COBS strain were maintained on purified diets (Table 1) differing in caloric source. A high fat diet (60% hydrogenated vegetable oil), a starch diet (77% corn starch), and a sucrose diet (77% sucrose) were available ad libitum. All diets were isocaloric for protein. Half the mice on each regimen received 0.13 mg of caffeine per calorie of diet; the controls received the diet without the additive. The mice were drinking tap water ad libitum which contained 5.68 mg/lOO ml. This chronic ingestion of caffeine continued for about 10 weeks. At the end of this period the mice were challenged with a glucose tolerance test as described above except half of the animals received 0.43 mg/100 ml caffeine in their glucose load. In vitro measurements of tissue metabolism were performed using standard manometric techniques. In the first series of experiments, liver slices, kidney slices, hemidiaphragm and minced fat pads were incubated in a Krebs buffer containing 5.68 mg/100 ml. The incubation contents of a side arm containing either insulin or insulin plus caffeine were emptied into the flask and oxygen consumption was observed for an additional 60 min. At the end of this incubation the contents of a side arm containing either insulin or insulin plus caffeine were emptied into the flask and oxygen consumption was observed for an additional 60 min. In a second series of experiments in vitro, the tissues were incubated in the buffer plus caffeine for 30 min at the end of which time the contents of the side arm containing either the same medium or the medium plus insulin and caffeine were added to the flask. Oxygen consumption was maintained for 45 min after the side arm was emptied. Upon completion of the incubation in both sets of experiments the tissues were analyzed for glycogen by the anthrone method and for protein by the Biuret technique. Tissues from another set of animals were analyzed for cholesterol content using an automated method.

RESULTS

Figure 1 presents the data from the glucose tolerance tests. While the decrease in glucose tolerance with the feeding of a high fat carbohydrate-free diet was expected,
Fig. 1—Glucose tolerance curves: Solid lines represent curve for blood glucose values from animals which were consuming the basal diet and dotted lines the curve for animals consuming the basal diet plus caffeine. Bars indicate SEM. There were 8 mice in each group: (A) Mice fed the starch diet; (B) Mice fed the fat diet; and (C) Mice fed the sucrose diet.

There was an even greater response by the sucrose fed mice. In each case the mice on the diets which included caffeine exhibited a greater ability to remove glucose from the blood than the control animals on the diet alone. The change is least in the mice on the fat diet and starch diet and the greatest with sucrose feeding. Figure 2 contains data from the second set of glucose tolerance tests which were performed. The laboratory chow diet fed to these mice resembles the starch diet in composition and the glucose tolerance curve of the control animals is similar to that found in the preceding experiment. When the glucose challenge contains caffeine, however, the response is a more rapid return of blood glucose to original values.

The data in Table 2 are from the in vivo test of glucose metabolism and insulin action. Since pool sizes of precursors were not determined in these animals under the various conditions of the experiment, the ratio of uptakes by diaphragm and adipose tissue was calculated. A high ratio could be indicative of greatly increased diaphragm uptake or of decreased adipose tissue utilization of glucose. The relatively slow removal of glucose from the blood would suggest the latter situation and rapid removal of the former. Inspection of the data in Table 2 reveals an inconsistent pattern. Fat feeding depresses the response to insulin by the adipose tissue but feeding the fat-plus-caffeine diet restores the insulin response as indicated by both the D/At ratios and the number of counts per milligram of dry weight for that tissue.

On the other hand, the starch fed mice show a clear response to insulin which is depressed when the diet includes caffeine. The data from the number of counts appear to indicate that lipogenesis is proceeding at a rapid rate in the absence of insulin and is exaggerated in response to that hormone. The metabolic pattern in the animals eating the sucrose diet shows no effect of the caffeine treatment.

The data presented in Tables 3 and 4 resulted from the in vitro measurements of metabolic activity. Since changes in metabolic pattern were more reproducible and of greater interest the data are comparisons of rates between samples of tissues from the same mouse. Percent stimulation was determined by the equation: 

\[
\% = \left( \frac{+SA}{-SA} \right) \times 100
\]

where the (-SA) is the amount of tissue glycogen accumulation or QO2 after addition of the control side arm's contents and (+SA) is the observation in the experimental situation. In the first test (Table 3) the liver of the chronic caffeine drinkers showed a lessened QO2 response to insulin if caffeine was simultaneously presented when compared to the water drinking animals. Lack of tissue differences in diaphragm and fat pad as measured by either QO2 or glycogen stores, decreases the utility of these observations. To ascertain the effect of a chronic level of caffeine in body fluids the data in Table 4 were obtained. In this situation the diaphragm of the chronic caffeine drinkers is able to respond to an insulin and glucose challenge by maintaining a higher glycogen level. The glycogen level occurs after pre-incubation with caffeine.

A measure of changed lipid metabolism was attempted by determination of tissue cholesterol levels as presented in Table 5. In no case was there a statistically significant difference between controls and chronic caffeine ingesters.

**DISCUSSION**

THE CHANGED ABILITY of the mice to respond to an oral glucose load can only be related to a basic metabolic adaptation to chronic caffeine ingestion. Reports of de-
creased clearance after acute ingestion of a caffeine solution can be explained as representing caffeine effects on cyclic AMP levels via a blocking action on degradation of the latter compounds by the enzyme phosphodiesterase (Sutherland, 1972). Since all food during the preceding 4 wk contained caffeine it can be hypothesized that a homeostatic equilibrium had occurred which allowed for the presence of the caffeine and an adjustment of the metabolic apparatus had occurred. The new equilibrium could be achieved by increased phosphodiesterase levels or by decreasing cyclic AMP production or by other possible mechanisms too numerous to speculate upon.

Care must be taken in proposing a single mechanism for the observed effect on glucose tolerance curves in view of the in vivo data. These data show a significant difference in rate between tissue from water and caffeine drinking group p < 0.05 as determined by the nonparametric Mann-Whitney test.

Upon that hormone for metabolic homeostasis. Both compounds-fuctose and caffeine-apparently would increase the amount of insulin released in response to a stimulus and thereby set into play mechanisms to maintain an equilibrium.

Thus, the chronic feeding of a diet containing caffeine causes a variety of adaptations. Effects reported after acute caffeine ingestion may not be applicable to the chronic situation. To differentiate among facilitated insulin release (Challes et al., 1973), increased steroid levels (Sutherland, 1972), metabolic adaptation and any possible combination of these circumstances, the second series of tests were performed. The only clear response observed in vitro was a greater glycogen level in the diaphragm of the chronic caffeine drinkers. In view of the findings in vivo this result can only be interpreted as indicating that the fat pad is at a high level of anabolism while the

Table 3—Percent caffeine stimulation of glycogenolysis

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Liver</th>
<th>Kidney</th>
<th>Diaphragm</th>
<th>Fat pad</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>-15a</td>
<td>-3</td>
<td>+12</td>
<td>-12</td>
</tr>
<tr>
<td>Caffeine</td>
<td>-9</td>
<td>-12</td>
<td>+11</td>
<td>-8</td>
</tr>
</tbody>
</table>

* Percent stimulation = [(+SA) - (-SA)]/(-SA) x 100 where -SA = side arm content of buffer only and +SA = side arm contents of buffer plus insulin and caffeine

b Significant difference is rate between tissue from water and caffeine drinking group p < 0.05 as determined by the nonparametric Mann-Whitney test.
**GROWTH OF C. perfringens ON α-GALACTOSIDES... From page 1760**


**REFERENCES**


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