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NUTRIENT INTERACTION IN RELATION TO GLYCAEMIC RESPONSE IN ISOCARBOHYDRATE AND ISOCALORIC MEALS

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Abstract : The present study was designed to examine the effect of casein (Cs) on postprandial glycaemia when ingested with glucose (G) alone or in combination with corn oil (Co), cellulose (Cl) or pectin (P). The study was conducted on a pool of ten healthy male volunteers in two sets of five volunteers each. The meals administered in the two sets were similar in composition but were isocarbohydrate (100 g G) in one set, and isocaloric (400 kcal) in another set. The meals in each set consisted of G, G Cs, G Cs Co, G Cs Cl and G Cs P. Each of the five volunteers in a given set underwent five meal tolerance tests (MTT), once with each meal, in a Latin Square design. During the MTT, the meal was administered after an overnight fast. In addition to a fasting venous blood sample, blood was collected 0.5, 1.0, 1.5 and 2.0 h after ingestion for measurement of serum glucose and insulin levels. In both sets, the highest glycaemic response was that to G. In the isocarbohydrate set, G Cs gave a significantly lower glycaemic and insulinaemic response than G. Further addition of Co made no essential difference but both the fibre containing meals gave significantly lower glycaemic responses. The insulinaemic response was attenuated only in case of G Cs P but not in case of G Cs Cl. In the isocaloric set, Cs as G Cs was observed to stimulate insulin secretion rather than attenuate postprandial glycaemia .G Cs Co gave a reduction in glycaemic as well as insulinaemic response as compared to G. Both fibre containing meals led to futher reduction in both responses, P being somewhat more effective than Cl. Addition of other nutrients to G, in general, reduces postprandial glycaemia.

Key words : Casein Glucose tolerance test Glycaemic index Postprandial glycaemia

INTRODUCTION

It is now generally agreed that reduction of postprandial glycaemia is a sound and rational goal for prevention and treatment of diabetes mellitus. This aim can be achieved by several means, of which alteration of nutrient composition is one (1). The nutrients which have been shown to reduce postprandial glycaemia include proteins (2-5), fats (2, 3, 6-8) and dietary fibre (3, 9-17).

Early studies by Estrich et al (2) had revealed that attenuation of glycaemia was more marked in the presence of both proteins and fat than in presence of either alone. Several other studies also support the observation that mixed meals give a markedly lower postprandial glycaemic response than carbohydrates alone (5, 18-20). Most of these studies were conducted in isocarbohydrate conditions while alternative diets normally designed for the normal population or diabetics are isocaloric.

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Therefore there is need for glycaemic response studies using isocaloric meals also.

The present study was designed to study the effect of coingestion of protein alone on the glycaemic response to glucose, and the effect of further addition of fat or fibre to the meal in isocarbohydrate or isocaloric combinations.

METHODS

Subjects: The study was conducted on ten healthy young human male volunters (age, 18-42 years; body weight 44-68 kg; height, 162-177 cm). The study was performed in two sets of five volunteers each.

The subjects were on their usual diet which provided at least 250 g carbohydrate every day. They were expected to abstain from late night snacks, smoking, and alcohol on the day preceding the meal tolerance test (MTT).

Ethical considerations: The protocol of the study was approved by the Ethics Committee of the All India Institute of Medical Sciences, New Delhi. An informed consent was obtained prior to enlisting a subject for the study.

Meals: The meals comprised of two sets of five meals each (Table 1). The constituents of the meals were identical in the isocarbohydrate and isocaloric sets. The 100 g oral glucose tolerance test (OGTT) was performed in both the sets for comparison with other meals. Except for this reference meal, all other meals had glucose and casein, with or without one or more other nutrient.

The meals were prepared on the morning of the test by hydration 0.5 h before ingestion. The meals were provided in a standardized 400 ml volume.

Meal Tolerance Tests: The volunteers were studied after an overnight fast on five mornings at weekly intervals. After a fasting venous blood sample had been drawn (before 10.00 AM), they were administered one of the five 'meals' in that set in accordance with a 5×5 Latin Square design. Each meal was consumed in 5-10 min at a steady rate. The mid-point between starting and finishing the meal was taken as zero time. Venous blood samples were drawn 0.5, 1.0 1.5 and 2.0 h after ingestion. Serum was separated within 0.5 h by clot breaking and centrifuging for 10 min at low speed.

Analysis: Each blood sample was analysed for serum glucose by the O-toluidine method and serum insulin by double antibody radioimmunoassay.

Calculations: From serial estimations of serum glucose and insulin, the folowing indices were derived: area under the 2-h glucose curve (AUC-G), area under the 2-h insulin curve (AUC-I), corresponding incremental areas ($\triangle$AUC-G and $\triangle$AUC-I), glycaemic index (GI) and insulinaemic index, and corresponding incremental indices, $\triangle$GI and $\triangle$Insulinalmic index.

Areas under the glucose and insulin curves were calculated by using a programmable calculator (Hewlett Packard 41 CV). The glycaemic index was calculated using the formula:

$$\text{Glycaemic Index} = \frac{\text{AUC-G in response to the meal}}{\text{AUC-G in response to 100 g glucose}} \times 100$$

Similarly the insulinaemic index was calculated using the formula:

$$\text{Insulinaemic Index} = \frac{\text{AUC-I in response to the meal}}{\text{AUC-I in response to 100 g glucose}} \times 100$$

For the incremental indices, corresponding incremental areas were used instead of the absolute areas.

Statistical analysis: The observed and computed parameters following different meals were compared by analysis of variance (ANOVA). The points at which a significant difference between meals could be expected on the basis of ANOVA analysis were subjected to Newman-Keuls' multiple range test. Newman-Keuls' test is a rather conservative multiple range test, and therefore sometimes misses even some fairly marked differences. To minimise the

chances of missing genuine differences, paired comparisons by Student's t-test were also made between each meal and the control (glucose meal). This was considered reasonable even in a multiple test situation because using the response to 100 g Glucose as the reference for comparison was built into the protocol of the study. Differences were considered significant when $p < 0.05$ but marginally significant results ($0.05 < p < 0.10$) have also been reported.

RESULTS

Isocarbohydrate meals: The glycaemic and insulinaemic responses to the meals are given in Figs. 1 and 2, and the computed indices in Table II.

The highest glycaemic and insulinaemic response was obtained after administration of glucose (G). Addition of casein (Cs) to glucose (G Cs) significantly lowered the glycaemia at 0.5 h ($p < 0.01$) as well as AUC-G ($p < 0.05$). The insulinaemic response to G Cs was also significantly lower than that to G at 1.0 h ($p < 0.05$). Further addition of corn oil (Co) neither altered the glycaemic response nor the insulinaemic response significantly. Both the fibre containing meals gave a significantly lower glycaemic response than G. The cellulose (Cl) containing meal G Cs Cl lowered postprandial glycaemia at 1.0 h ($p < 0.05$) and 1.5 h ($p < 0.1$), and also the AUC-G ($p < 0.05$). The pectin (P) containing meal (G Cs P) lowered

TABLE I : Composition of the Experimental Meals.

Meal	*G (g)*	*Cs (g)*	*Co (g)*	*Cl (g)*	*P (g)*	*Energy (kcal)*
Isocarbohydrate set						
1. G	100	—	—	—	—	400
2. GCs	100	20	—	—	—	480
3. G Cs Co	100	20	9	—	—	560
4. G Cs Cl	100	20	—	20	—	480
5. G Cs P	100	20	—	—	20	480
Isocaloric set.						
1. G	100	—	—	—	—	400
2. G Cs	60	40	—	—	—	400
3. G Cs Co	60	20	9	—	—	400
4. G Cs Cl	60	40	—	20	—	400
5. G Cs P	60	40	—	20	—	400

G, glucose; Cs casein; Co, corn oil; Cl, cellulose; P, pectin

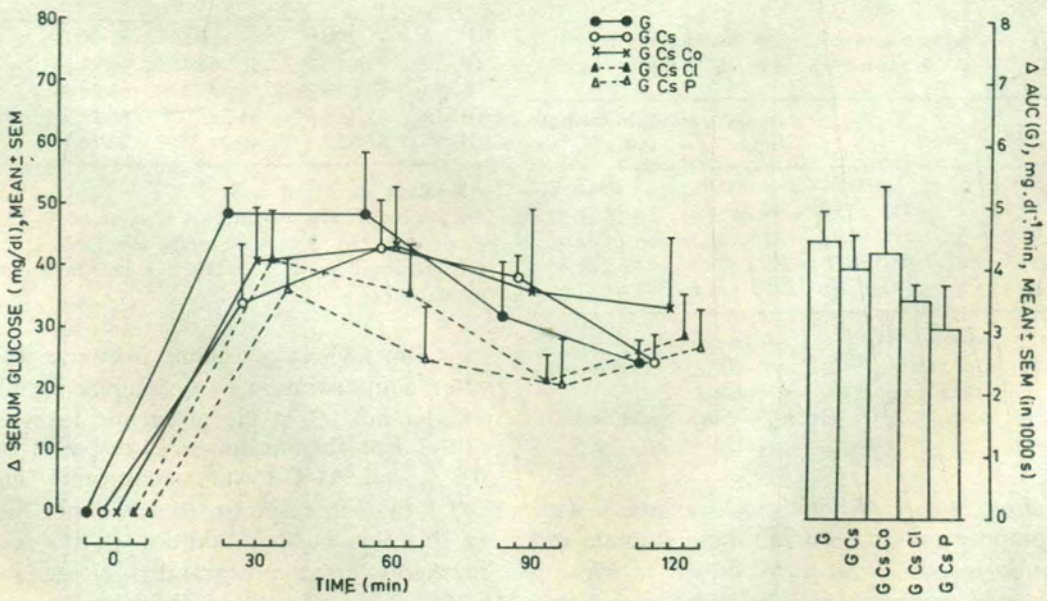

Fig. 1: Serum glucose response to the isocarbohydrate meals administered.

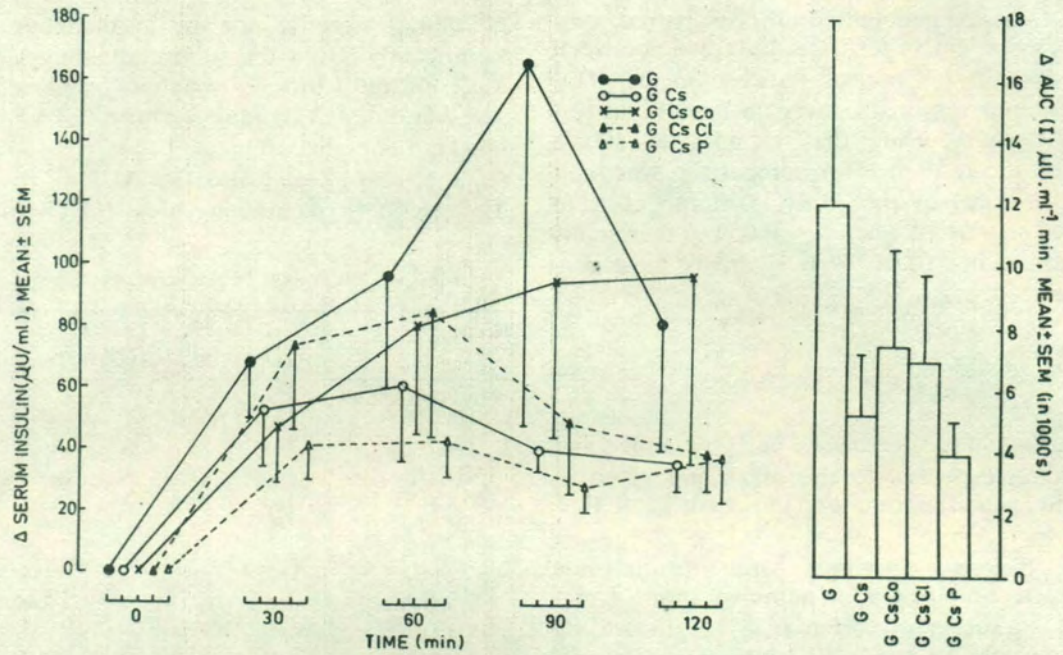

Fig. 2: Serum insulin response to the isocarbohydrate meals administered.

the glycaemia at 1.0 h ($p < 0.05$) and 1.5 h ($p < 0.01$), and also the AUC-G ($p < 0.05$), and also gave the lowest glycaemic and insulinaemic indices (Table II).

TABLE II : Indices of glycaemic and insulinaemic rersponse to the isocarbohydrate meals tested.

Meal	*GI*	△GI	Insulinaemic Index	△Insulinaemic index
G	100.0	100.0	100.0	100.0
GCs	90.8±2.7	93.4±15.4	74.0±18.3	75.7±21.8
GCsCo	97.2±7.5	91.7±18.4	84.9±20.5	86.7±25.6
GCsCl	89.3±5.4	80.4±5.3	85.2±18.8	81.7±21.8
GCsP	85.4±5.6	67.8±10.9	74.0±31.0	71.4±37.6

All values are Mean ± SE.
G, glucose; Cs, casein; Co, corn oil; Cl, cellulose; P, pecin; for details of meals, see Table I.
GI, glycaemic index; △ GI, glycaemic index based on incremental areas

Isocaloric meals: When isocaloric meals with similar composition were studied the glycaemic and insulinaemic responses were as shown in Figs. 3 and 4, and the computed indices as given in Table III.

TABLE III : Indices of glycaemic and insulinaemic response to the isocaloric meals tested.

Meal	*GI*	△GI	Insulinaemic Index	△Insulinaemic index
G	100.0	100.0	100.0	100.0
GCs	98.9±8.5	102.6±15.7	108.4±31.2	111.5±35.6
GCsCo	93.8±4.2	56.8±13.8	62.2±13.0	55.9±13.6
GCsCl	85.7±6.9	51.2±20.7	65.1±9.9	62.1±10.5
GCsP	87.6±3.3	48.2±8.0	40.1±14.5	32.5±14.8

All values are Mean ± SE.
G, glucose; Cs, casein; Co, corn oil; Cl, cellulose; P, pectin; for details of meals, see Table I.
GI, glycaemic index; △ GI, glycaemic index based on incremental areas

The highest glycaemic response was obtained after administration of G. Replacing 40 g G with Cs did not affect the glycaemic response significantly. But the insulinaemic response to G Cs at 0.5 h and AUC-I were significantly higher ($p < 0.05$) than in response to any other meal studied in this set. Further addition of Co resulted in a markedly lower postprandial glycaemia at 0.5 h and 1.0 h as well as insulinaemia at 0.5 h as compared to G as well as G Cs. The fibre containing

meals (G Cs Cl and G Cs P) gave a significantly lower incremental glycaemia at 0.5 h as compared to G or G Cs. Absolute and incremental insulin levels at 0.5 h and AUC-I were also significantly lower in response to G Cs or G Cs P as compared to G or G Cs. The glycaemic and insulinaemic indices were the lowest with G Cs P as in case of isocarbohydrate meals (Table III).

DISCUSSION

The study was designed primarily to investigate

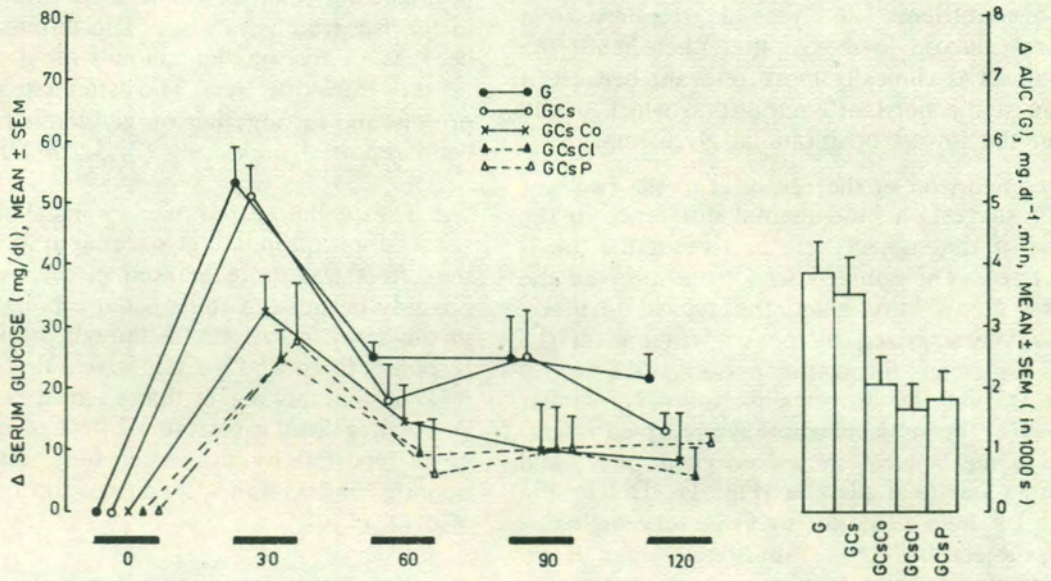

Fig. 3: Serum glucose response to the isocaloric meals administered.

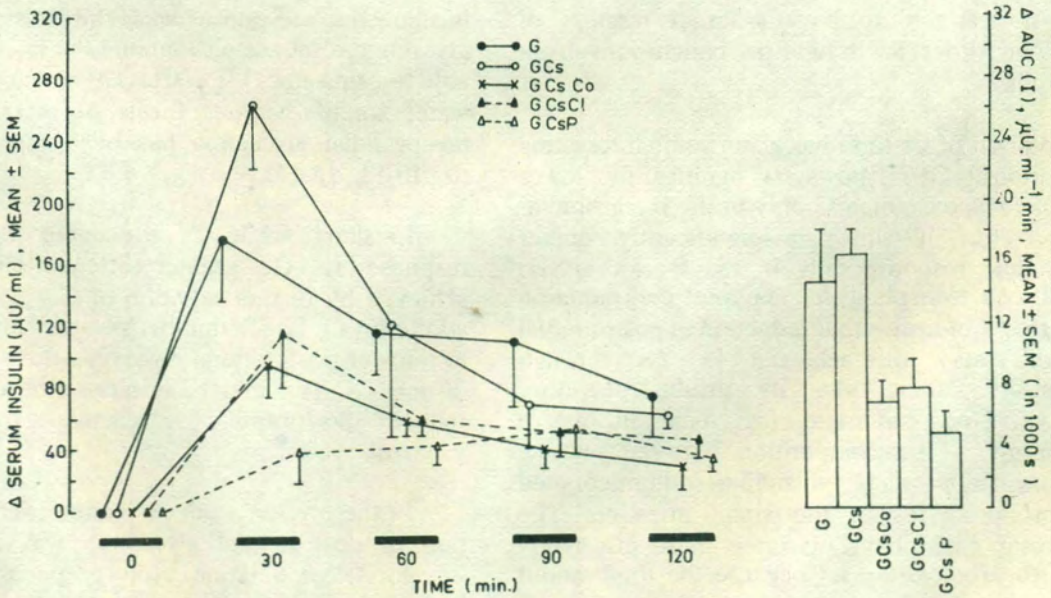

Fig. 4: Serum insulin to the isocaloric meals administered.

how addition of Cs influenced the glycaemic response to G. The effects of further addition of Co, Cl or P to the carbohydrate and protein containing meal have also been studied. The meals were grouped into two sets with different objectives in mind. The isocarbohydrate set is physiologically more relevant because it demonstrates the effect of various nutrients on glycaemic response to a fixed oral glucose load. On the other hand, the isocaloric set is clinically more relevant because it can suggest the nutrient composition which would result in the lowest postprandial glycaemia.

A comparison of the response to the two sets of meals suggests a fundamental difference in the response of the subjects irrespective of the meals administered. The subjects who have received the isocaloric meals have given the typical textbook response characterized by peak glycaemia at 0.5 h, and a plateau during the period 1.0 h to 2.0 h (Fig. 3). The insulin response follows a similar pattern. But the subjects who have received isocarbohydrate meals have shown no clear peak, and no distinct fall to a plateau (Fig. 1). The insulin level at 1.5 h in response to G is very high due to one subject having an abnormally high level. The response in the two groups of apparently healthy subjects illustrates the broad range of normal response. Such large individual variation makes it desirable to have a larger number of subjects in order to draw firm conclusions from such studies.

Addition of Cs to G has given some interesting observations. GCs shows a significantly lower glycaemic response than G only in the isocarbohydrate set but it shows a significantly higher insulinaemic response only in the isocaloric set. It is difficult to explain this apparent contradiction except that it indicates that reduction in postprandial glycaemia may be achieved by Cs through mechanisms other than its insulin secretory response. One candidate for such alternative mechanisms is the competition between glucose and some amino acids for similar sodium-coupled transport processes in the small intestine. The mechanism might have operated more effectively in the isocarbohydrate set because the total amout of glucose to be absorbed was greater than in the isocaloric set. Why the insulin secretory response to GCs is not accompanied by a reduction in postprandial glycaemia is difficult to explain. However, the insulin secretory response to proteins has been reported earlier (4, 21).

The corn oil containing meal (G Cs Co) gave a significantly lower postprandial glycaemia and insulinaemia than G in the isocaloric set but not in the isocarbohydrate set. The difference may be because of the smaller amount of G in the meal in the isocaloric set. The attenuating effect of protein and fat together on glycaemic response has been reported previously (2, 5, 18-20).

The cellulose containing meal (G Cs Cl) reduced postprandial glycaemia in both sets but the effect was more marked in the isocaloric set, possibly because of the smaller amount of glucose in the meal in this set. Although the insulinaemic response to to G Cs Cl is lower than that to G, it is considerably higher than to most meals studied. A possible insulin secretory effect of cellulose has been reported by us earlier (16), and seems to operate under some conditions in other studies also (22).

The pectin containing meal (G Cs P) gave the lowest glycaemic and insulinaemic response in the isocarbohydrate set. In the isocaloric set, its insulinaemic response was the lowest, and its glycaemic response also among the lowest, comparable to that to G Cs Cl. The marked effect of water soluble viscous forms of dietary fibre on postprandial glycaemia has been reported earlier (3, 10-12, 15-17).

In short, while Cs attenuated the glycaemic response to G, greater attenuation could be achieved by further addition of Co, Cl or P. Thus addition of a larger number of nutrients to G leads to a lower postprandial glycaemia than the addition of only Cs. This may be because different nutrients reduce postprandial glycaemia by different mechanisms.

In the present study, in general, greater reduction in postprandial glycaemia was achieved by isocaloric combinations (60 g glucose) than by isocarbohydrate (100 g glucose) combinations (Table IV). There are a few studies available which

TABLE IV: Comparative postprandial glycaemia and insulinaemia in response to meals studies.

Meals Compared	*% change in △AIG-G*		*% change in △AUC-I*	
	Isocarbo-hydrate meals	*Isocal-oric meals*	*Isocarbo-hydrate meals*	*Isocal-oric meals*
G vs. G Cs	−9.9	−8.9	−56.3	+12.3
G vs. G Cs Co	−4.0	−47.0	−38.2	−53.3
G vs. G Cs Cl	−20.9	−56.9	−42.2	−45.8
G vs. G Cs P	−30.6	−53.8	−67.1	−67.0

△AUC-G, incremental area under the 2-h glucose curve; △AUC-I, incremental area under the 2-h insulin curve.
G, glucose; Cs, casein; Co, corn oil; Cl, cellulose; P, pectin
For details of meals, see Table I.

have compared the glycaemic response to different doses of oral glucose ranging between 50 and 100 g (23-27). In general, the postprandial glycaemia is about 20 mg/dl higher at 1.0 h and 2.0 h with the higher doses. The reductions in glycaemia observed in the present study in this isocaloric set are of a greater magnitude, and occur principally at 0.5 h. Hence the attenuation of glycaemic response to G by the added nutrients is unlikely to be wholly due to the lower dose of G in the meal. Most of the earlier studies on coingestion of nutrients have been under isocarbohydrate conditions. The present study indicates that the principles derived from studies with isocarbohydrate meals can be extrapolated to the isolcaloric diabetic diets. In fact, the effects of nutrients which reduce postprandial glycaemia are magnified in isocaloric meals because of the simultaneous reduction in the amount of carbohydrate.

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