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Fasting and postprandial lipid and glucose metabolisms are modulated by dietary proteins and carbohydrates: Role of plasma insulin concentrations

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The purpose of this study was to verify the respective and interactive effects of dietary proteins and carbohydrates on lipid and glucose metabolisms. In order to do so, 120 male Sprague–Dawley rats were fed low-fat high-carbohydrate purified diets varying in both protein (20%) and carbohydrate (60%) sources consisting of either casein-cornstarch, casein-sucrose, soy protein-cornstarch, soy protein-sucrose, cod protein-cornstarch, or cod protein-sucrose for 28 days. In fasted and fed rats, casein induced a hypercholesterolemic effect compared with soy or cod protein. However, on hepatic lipids, cod protein responded similarly to casein, being more elevated than soy protein. The independent carbohydrate effect was seen by decreased levels of liver cholesterol and triglycerides with sucrose diets compared with cornstarch diets. An interaction was also observed between dietary proteins and carbohydrates, which produced higher postprandial insulin levels with cod protein-cornstarch and casein-sucrose diets, suggesting that different mechanisms are involved in the insulin secretion of rats fed cod protein and casein. It is therefore possible that dietary proteins and carbohydrates may influence fasting and postprandial lipid and glucose metabolisms through plasma insulin levels. (J. Nutr. Biochem. 6: 540–546, 1995.)

Keywords: dietary proteins; dietary carbohydrates; plasma lipids, hepatic lipids; insulin

Introduction

Animal proteins are known to be hypercholesterolemic compared with plant proteins.[1] Rats and rabbits fed casein generally result in higher serum cholesterol levels than those fed soy protein.[2,3] However, the magnitude of this cholesterolemic response to dietary proteins is influenced by the type of carbohydrate included in the diet.[4] It has been shown that dietary sucrose but not dietary cornstarch promotes a casein-induced hypercholesterolemia in rats.[4]

Fish protein is now being used to study the effects of other animal proteins on cholesterol metabolism. Feeding fish protein in a cholesterol-free semipurified diet containing cornstarch exerted intermediate but not significantly different serum cholesterol levels to casein and soy protein in rats and rabbits.[5,6] By contrast, when fish protein was included in a cholesterol-free semipurified diet containing sucrose, a hypocholesterolemic effect similar to that of soy protein was observed in rats with respect to casein.[7] Kritchevsky et al.[3] demonstrated a hypocholesterolemic effect of fish protein in rabbits also in comparison with casein when combined with a high-sucrose diet. The variations observed in serum cholesterol levels when rats are fed fish protein combined with different carbohydrate sources suggest that proteins and carbohydrates may act together in the regulation of cholesterol metabolism.

Elevation in serum triglycerides has been observed in rats fed sucrose-rich diets.[8] Comparative studies made of either sucrose- or starch-containing diets demonstrated that the sucrose-induced hypertriglyceridemia resulted primarily from increased hepatic lipogenic capacity.[9] That sucrose is an insulin secretagogue is already well known, as reported

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by Reaven et al.,[10] and this elevated secretion is probably in relation with the sucrose stimulation of lipogenic enzyme activity.[8]

The origin of dietary proteins also appears to be a modulator of insulin and glucagon secretion. Casein as compared with soy protein has been shown to cause an increase in the concentration of serum insulin levels of rats, whereas plasma glucagon remained unchanged.[2] In humans, blood insulin levels also increased following a casein meal versus a soy protein meal, although glucagon levels seemed to decrease.[11] In another experiment with rats, Galibois et al.[12] demonstrated different responses on plasma insulin and glucose to dietary proteins when in the fasting or postprandial state. In fasted rats, plasma glucose but not insulin was higher in those fed casein than those fed soy protein, whereas in the fed state both the glycemic and insulinemic responses were stimulated, again giving higher levels with casein than soy protein.

In order to assess the independent and interactive effects of dietary proteins and carbohydrates on lipid and glucose metabolisms, we designed the present study by means of a 3 × 2 factorial model. To do so, rats were fed purified diets differing in casein, soy protein, or cod protein combined with either cornstarch or sucrose. We also explored whether the expected changes are insulin- and glucagon-dependent. All measurements in plasma were done both in fasted and fed states, whereas hepatic values were obtained in the fasted state only.

Methods and materials

Experimental animals

One hundred and twenty Sprague–Dawley rats (St. Constant, Québec, Canada) initially weighing approximately 230 g were housed individually in stainless steel wire-bottomed cages placed in an animal room at constant temperature (20 to 24°C) and humidity (45 to 55%) and kept under a daily dephased light-dark cycle (0700 to 1900). Upon arrival, all rats were fed a nonpurified commercial diet (Purina rat chow; Ralston Purina Inc., Lasalle, Québec, Canada) for 2 days. At the end of this adaptation period, the rats were divided according to their weight into 6 groups of 10 rats which would be sacrificed in the fasted state, and 6 other groups of 10 rats to be sacrificed in the fed state. Purified diets and water were provided ad libitum. Records of food intake and body weight were taken three times a week. This protocol was approved by the animal ethics committee of Université Laval.

Purified diets

The purified diets varying in both protein and carbohydrate sources consisted of either casein-cornstarch (CA-CS), casein-sucrose (CA-SU), soy protein-cornstarch (SP-CS), soy protein-sucrose (SP-SU), cod protein-cornstarch (CP-CS), or cod protein-sucrose (CP-SU). The composition of these diets is shown in *Table 1*. The cod protein was prepared in our laboratory by lyophilization of frozen cod fillets which were delipidated in an industrial Soxhlet-type apparatus for 24 hr using diethyl ether as a solvent. The residual lipid content of casein (0.03%), soy protein (0.4%), and cod protein (0.1%) was verified with a Goldfisch Lipid Extractor (Model 35001; Labconco Corporation, Kansas City, MO USA). The energy content of the diets was measured in an automatic adiabatic calorimeter (Model 1241; Parr Instruments, Moline, IL USA) and were found to be isoenergetic: casein-cornstarch (18.99 kJ/g), casein-sucrose (19.04 kJ/g), soy protein-cornstarch (18.79 kJ/g), soy protein-sucrose (18.80 kJ/g), cod protein-cornstarch (18.57 kJ/g), and cod protein-sucrose (18.74 kJ/g). The protein content (N × 6.25) was assayed by Kjeldahl-Foss autoanalyzer (Model 16210; Foss Co., Hillerod, Denmark). The level of protein in the purified diets was adjusted to an isonitrogenous basis at the expense of the carbohydrates.

At the end of the 28-day experimental period, all rats were starved for 16 hr and 60 of them were then fed a similar purified

Table 1 Composition of the purified diets[a]

Ingredient	CA-CS (g/100 g)	CA-SU (g/100 g)	SP-CS (g/100 g)	SP-SU (g/100 g)	CP-CS (g/100 g)	CP-SU (g/100 g)
Casein[b]	22.5	22.5	—	—	—	—
Soy protein[c]	—	—	22.6	22.6	—	—
Cod protein[d]	—	—	—	—	21.7	21.7
Cornstarch[e]	58.8	—	58.7	—	59.6	—
Sucrose[e]	—	58.8	—	58.7	—	59.6
Cellulose[f]	5	5	5	5	5	5
Coconut oil[g]	7	7	7	7	7	7
Corn oil[h]	1	1	1	1	1	1
Cholesterol[g]	1	1	1	1	1	1
Minerals[i]	3.5	3.5	3.5	3.5	3.5	3.5
Vitamins[j]	1	1	1	1	1	1
Choline bitartrate[e]	0.2	0.2	0.2	0.2	0.2	0.2

[a]CA = casein; SP = soy protein; CP = cod protein; CS = cornstarch; SU = sucrose.
[b]Casein purified high nitrogen (ICN Nutritional Biochemicals, Cleveland, OH, USA) 88.6% protein.
[c]Soybean protein isolate, ICN 88.3% protein.
[d]92.0% protein.
[e]ICN Nutritional Biochemicals.
[f]Alphacel nonnutritive bulk (ICN Nutritional Biochemicals).
[g]Sigma Chemical Co. (St. Louis, MO, USA).
[h]Mazola corn oil, Best Foods (Canada Starch, Montreal, Canada).
[i]AIN mixture 76 (ICN Nutritional Biochemicals).
[j]Vitamin mix (Teklad Test Diets, Madison, WI, USA).

diet containing no coconut oil, no corn oil, and no cholesterol for 3 hr in order to avoid intestinal chylomicron formation. They were then weighed and killed by decapitation. The other 60 rats were weighed and sacrificed after the 16-hr fast.

Plasma lipoproteins and hepatic lipids

Blood samples of fed rats were collected in a 10 mL tube containing 12.5 mg of EDTA and centrifuged at 3,000 rpm at 4°C in order to obtain plasma. Cholesterol and triglycerides were determined enzymatically with kits provided by Boehringer Mannheim (Boehringer Mannheim Canada, Laval, Québec). High-density lipoprotein cholesterol (HDL-C) was determined enzymatically after precipitation of low-density lipoproteins (LDL) and very low-density lipoproteins (VLDL) with phosphotungstic acid and magnesium ions as described by Burnstein et al.[13]

Blood samples of fasted rats were also collected with 10 mL tubes plus EDTA and centrifuged at 3,000 rpm at 4°C in order to obtain plasma. Livers were removed, weighed, frozen in liquid nitrogen, and stored at −70°C until the extraction of hepatic lipids by chloroform: methanol (2:1, vol/vol) using the method of Folch et al.[14] Plasma and liver lipids were determined enzymatically as described previously.

Plasma glucose, insulin, and glucagon

Glucagon[15] and insulin[16] were measured by radioimmunoassay at the Diabetes Unit of Laval University Hospital and plasma glucose in our laboratory by means of a Technicon autoanalyzer (YSI 2700 Select; Terochem Scientific, Toronto, Canada) in all fasted and fed rats. Hepatic glycogen was determined with amyloglucosidase according to the method of Keppler and Decker.[17]

Statistical analysis

All calculations were performed with the Statistical Analysis System (SAS Institute, Cary, NC USA). Data were subjected to an analysis of variance (ANOVA) according to a 3 × 2 factorial arrangement, using the general linear model (GLM) procedure, in order to determine the main protein and carbohydrate effects as well as interactions between dietary protein and carbohydrate sources at $P < 0.05$. A Duncan's New-Multiple-Range test was applied when statistically significant interactions were detected in order to identify differences among diet groups. Data for hepatic glycogen were logarithmically transformed before statistical analysis to make comparisons among homogeneous means. However, glycogen values presented in tables are the untransformed means ± SEM.[18]

Results

The group of rats sacrificed in the fasted state as well as those sacrificed in the fed state all had a fairly similar food intake, body weight gain, and gross weight gain efficiency ratio between the dietary groups (*Table 2*). Food intake at the last meal was also similar between the six diet groups of the fed state rats.

Mean plasma total cholesterol, triglycerides, HDL-cholesterol (HDL-C) and hepatic cholesterol and triglycerides of fasted rats are shown in *Table 3*. The bottom half of the table shows the overall analysis of variance as well as multiple comparisons. As indicated by a significant P value ($P < 0.05$), dietary proteins and carbohydrates independently affected fasted plasma and hepatic lipids. In the fasted rats, casein compared with cod protein induced higher levels of total plasma cholesterol, triglycerides, and HDL-C. Casein also induced higher levels of total cholesterol when compared with soy protein. Significantly higher levels of hepatic cholesterol and triglycerides were observed when rats were fed casein and cod protein compared with soy protein. It is interesting to note that cod protein induced a cholesterolemia similar to soy protein, but when looking at liver lipids cod protein led to higher lipid levels such as casein. Carbohydrates independently affected hepatic cholesterol and triglycerides. Sucrose significantly decreased the levels of cholesterol and triglycerides in the liver compared with cornstarch.

Table 4 shows the mean plasma glucose, insulin, glucagon, insulin to glucagon ratio, and hepatic glycogen for the rats sacrificed in the fasted state. The only protein effect

Table 2 Food intake, weight gain, and gross weight gain efficiency ratio (GWGE) of rats fed the purified diets[a]

Dietary[b] group	n	Food intake (g/day/animal)	Food intake last meal (g/animal)	Weight gain (g/day/animal)	GWGE[c]
Fasted					
CA-CS	10	23 ± 0.6		6.8 ± 0.2	0.30 ± 0.01
CA-SU	10	23 ± 0.4		7.2 ± 0.2	0.31 ± 0.01
SP-CS	8	21 ± 0.3		6.2 ± 0.2	0.29 ± 0.01
SP-SU	10	23 ± 0.6	NA	6.9 ± 0.3	0.30 ± 0.01
CP-CS	10	22 ± 0.6		6.8 ± 0.2	0.31 ± 0.01
CP-SU	10	21 ± 0.5		6.7 ± 0.3	0.32 ± 0.01
Fed					
CA-CS	10	22 ± 0.8	7.2 ± 0.6	6.7 ± 0.4	0.30 ± 0.01
CA-SU	10	23 ± 1.0	7.2 ± 0.7	7.1 ± 0.4	0.31 ± 0.01
SP-CS	10	21 ± 0.4	5.6 ± 0.5	6.0 ± 0.2	0.29 ± 0.01
SP-SU	10	23 ± 0.7	6.2 ± 0.5	7.0 ± 0.4	0.31 ± 0.01
CP-CS	10	22 ± 0.5	6.3 ± 0.6	6.8 ± 0.2	0.31 ± 0.01
CP-SU	10	23 ± 0.7	6.6 ± 0.9	7.0 ± 0.3	0.31 ± 0.01

[a]Values are means ± SEM.
[b]CA = casein; SP = soy protein; CP = cod protein; CS = cornstarch; SU = sucrose.
[c]GWGE = weight gain (g/day/animal)/food intake (g/day/animal).

Table 3 Mean plasma total cholesterol, triglycerides, HDL-cholesterol, and hepatic cholesterol and triglycerides in fasted rats fed the purified diets[a]

Dietary[b] group	Cholesterol (mmol/L)	Triglycerides (mmol/L)	HDL-C (mmol/L)	Chol-HEP (μmol/g)	TG-HEP (μmol/g)
CA-CS	5.5 ± 0.8	2.3 ± 0.4	3.0 ± 0.5	41 ± 2	50 ± 4
CA-SU	6.0 ± 0.7	2.5 ± 0.4	4.3 ± 0.9	31 ± 4	42 ± 6
SP-CS	3.4 ± 0.4	1.5 ± 0.1	2.1 ± 0.4	25 ± 3	33 ± 3
SP-SU	3.3 ± 0.4	2.2 ± 0.4	2.9 ± 0.6	20 ± 4	25 ± 5
CP-CS	4.2 ± 0.7	1.2 ± 0.1	2.4 ± 0.4	43 ± 5	49 ± 8
CP-SU	4.6 ± 0.6	1.6 ± 0.3	2.2 ± 0.2	28 ± 5	34 ± 5
ANOVA, P[c]					
Protein (P)	0.002	0.01	0.047	0.002	0.01
Carbohydrate (C)	0.59	0.08	0.18	0.003	0.03
P × C	0.89	0.66	0.39	0.55	0.75
Comparisons[d]					
Protein	CA > SP	CA = SP	CA = SP	CA > SP	CA > SP
	CA > CP	CA > CP	CA > CP	CA = CP	CA = CP
	SP = CP	SP = CP	SP = CP	SP < CP	SP < CP
Carbohydrate	CS = SU	CS = SU	CS = SU	CS > SU	CS > SU

[a]Values are means ± SEM.
[b]CA = casein; SP = soy protein; CP = cod protein; CS = cornstarch; SU = sucrose.
[c]$P < 0.05$ indicates significant effects.
[d]The symbols designate: =, no difference among the groups at $P < 0.05$; >, significantly higher than the group with which it is compared at $P < 0.05$; <, significantly lower than the group with which it is compared at $P < 0.05$.

observed was the increase of the plasma glucose level with casein compared with soy and cod protein. However, feeding sucrose compared with cornstarch significantly increased plasma glucose and insulin levels as well as the insulin to glucagon ratio.

In the fed state (*Table 5*), plasma total cholesterol, triglycerides, and HDL-C levels were also elevated by casein compared with soy or cod protein. The carbohydrate effect was shown by a significant increase of plasma total triglycerides by the sucrose diets in relation to cornstarch. Also, it is important to note that the insulin to glucagon ratio was higher in rats fed cod protein than in those fed soy protein. No significant difference was observed in plasma glucose, glucagon, nor in hepatic glycogen.

A significant interaction between proteins and carbohydrates in plasma insulin for the group of rats in the fed state is shown in *Figure 1*. Cod protein combined with cornstarch had significantly higher plasma insulin levels compared with cod protein-sucrose, casein-sucrose, soy protein-sucrose, and soy protein-cornstarch. Interestingly, although nonsignificant, casein-sucrose combined diet increased plasma insulin levels compared with casein-cornstarch combined diet. Rats fed either cod protein-cornstarch or casein-sucrose diet had significantly higher plasma insulin levels

Table 4 Mean hepatic glycogen and plasma glucose, insulin, glucagon, and insulin to glucagon ratio of fasted rats fed the purified diets[a]

Dietary[b] group	Glucose (mmol/L)	Insulin (pmol/L)	Glucagon (pg/mL)	Insulin/ glucagon	Glycogen (μmol/g)
CA-CS	7.8 ± 0.2	301 ± 34	139 ± 10	2.2 ± 0.2	20 ± 6
CA-SU	7.9 ± 0.1	367 ± 48	129 ± 6	3.0 ± 0.4	9 ± 3
SP-CS	6.8 ± 0.1	236 ± 28	156 ± 15	1.7 ± 0.3	9 ± 2
SP-SU	7.5 ± 0.3	327 ± 30	129 ± 16	2.8 ± 0.4	9 ± 1
CP-CS	7.1 ± 0.2	234 ± 29	123 ± 5	2.0 ± 0.3	9 ± 3
CP-SU	7.4 ± 0.2	301 ± 28	116 ± 10	2.7 ± 0.3	9 ± 3
ANOVA, P[c]					
Protein (P)	0.03	0.12	0.11	0.53	0.17
Carbohydrate (C)	0.003	0.01	0.12	0.002	0.28
P × C	0.66	0.92	0.65	0.82	0.19
Comparisons[d]					
Protein	CA > SP	CA = SP	CA = SP	CA = SP	CA = SP
	CA > CP	CA = CP	CA = CP	CA = CP	CA = CP
	SP = CP	SP = CP	SP = CP	SP = CP	SP = CP
Carboyhdrate	CS < SU	CS < SU	CS = SU	CS < SU	CS = SU

[a]Values are means ± SEM.
[b]CA = casein; SP = soy protein; CP = cod protein; CS = cornstarch; SU = sucrose.
[c]$P < 0.05$ indicates significant effects.
[d]The symbols designate: =, no difference among the groups at $P < 0.05$; >, significantly higher than the group with which it is compared at $P < 0.05$; <, significantly lower than the group with which it is compared at $P < 0.05$.

Table 5 Mean hepatic glycogen and plasma total cholesterol, triglyceride, HDL-cholesterol, glucose, glucagon, and insulin to glucagon ratio in rats in the fed state[a]

Dietary[b] group	Cholesterol (mmol/L)	Triglycerides (mmol/L)	HDL-C (mmol/L)	Glucose (mmol/L)	Glucagon (pg/mL)	Insulin/ glucagon	Glycogen (μmol/g)
CA-CS	4.1 ± 0.5	2.0 ± 0.3	3.0 ± 0.4	8.3 ± 0.1	170 ± 19	6.4 ± 1.1	33 ± 12
CA-SU	5.6 ± 0.7	3.5 ± 0.6	2.9 ± 0.4	8.3 ± 0.2	165 ± 16	7.9 ± 0.9	47 ± 13
SP-CS	2.8 ± 0.4	1.4 ± 0.3	2.3 ± 0.2	8.3 ± 0.2	175 ± 13	4.9 ± 0.6	30 ± 12
SP-SU	3.0 ± 0.5	2.4 ± 0.4	2.3 ± 0.2	8.3 ± 0.1	148 ± 9	5.8 ± 0.5	35 ± 12
CP-CS	3.1 ± 0.4	1.3 ± 0.4	2.5 ± 0.2	8.3 ± 0.1	160 ± 9	8.6 ± 1.1	32 ± 13
CP-SU	2.8 ± 0.4	1.8 ± 0.3	2.1 ± 0.3	7.8 ± 0.2	131 ± 10	7.3 ± 1.4	40 ± 13
ANOVA, P[c]							
Protein (P)	0.0002	0.01	0.04	0.67	0.24	0.03	0.86
Carbohydrate (C)	0.29	0.003	0.47	0.64	0.07	0.67	0.35
P × C	0.16	0.41	0.78	0.33	0.59	0.36	0.95
Comparisons[d]							
Protein	CA > SP	CA > SP	CA > SP	CA = SP	CA = SP	CA = SP	CA = SP
	CA > CP	CA > CP	CA > CP	CA = CP	CA = CP	CA = CP	CA = CP
	SP = CP	SP = CP	SP = CP	SP = CP	SP = CP	SP < CP	SP = CP
Carbohydrate	CS = SU	CS < SU	CS = SU	CS = SU	CS = SU	CS = SU	CS = SU

[a]Values are means ± SEM.
[b]CA = casein; SP = soy protein; CP = cod protein; CS = cornstarch; SU = sucrose.
[c]$P < 0.05$ indicates significant effects.
[d]The symbols designate: =, no difference among the groups at $P < 0.05$; >, significantly higher than the group with which it is compared at $P < 0.05$; <, significantly lower than the group with which it is compared at $P < 0.05$.

than those fed soy protein-cornstarch or soy protein-sucrose. Plasma insulin was unaffected by the origin of carbohydrates in the presence of soy protein. The higher insulin to glucagon ratio observed with cod protein compared with soy protein (*Table 5*) is probably due to the higher insulin levels induced by the cod protein-cornstarch diet in the fed rats.

Discussion

Results from this study support the notion of casein being hypercholesterolemic compared with soy protein in rats.[2]

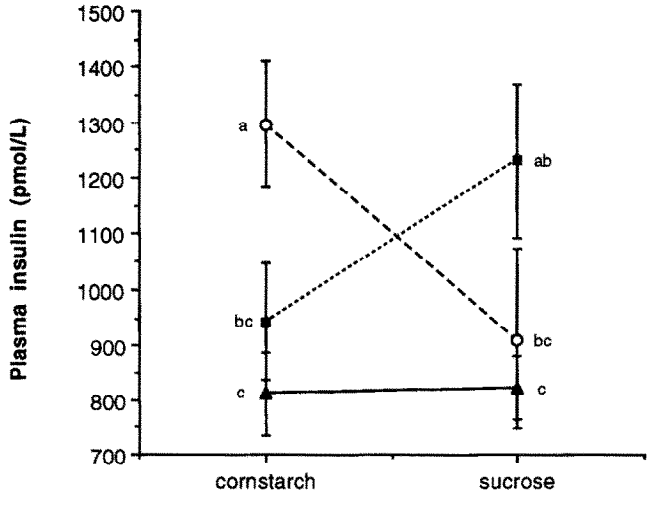

Figure 1 Interaction between dietary proteins and carbohydrates on postprandial plasma insulin levels in rats fed the purified diets. Values are expressed as means ± SEM. Groups bearing a similar letter are not significantly different ($P < 0.05$). --■-- casein; —○— cod protein; —▲— soy protein.

This increase of plasma cholesterol was reflected by an elevation of cholesterol in the HDL fraction in the fed state, also observed by Galibois et al.[12] This increase was also accompanied by higher postprandial triglyceride levels. Still consistent with prior observations, hepatic cholesterol and triglycerides increased with casein.[2] These results suggest that the rises in plasma and hepatic cholesterol and triglycerides on casein diets as compared with soy protein diets are probably related to an increased cholesterol intestinal absorption and a decreased excretion of bile acids and neutral steroids in feces.[19] Indeed the hyperlipidemia normally observed in a low-fat high-carbohydrate diet containing casein is usually prevented by soy protein.[4] The present data also demonstrated that casein compared with soy protein increased fasting plasma glucose. Moreover, when combined with sucrose, casein as compared with soy protein increased postprandial insulin concentrations and tended to elevate fasting insulin concentrations. These results suggest that such insulin response to an elevation of plasma glucose is probably a compensatory increase of insulin secretion which could be eventually followed long-term by increased day-long insulin concentrations, suggesting a loss of glucose tolerance. Furthermore, it has been reported that dietary sucrose versus starch leads to impairment of insulin action through the liver.[20] However, it appears from the present results that this hyperinsulinemic response is present when sucrose is combined with casein which has been shown to elevate plasma fasting glucose and postprandial insulin levels[12] but not when sucrose is combined with soy or cod protein.

Indeed hyperinsulinemia has often been correlated to increased plasma total triglyceride concentrations, which in humans may predispose to the development of cardiovascular diseases when associated with lower plasma HLD-C concentrations.[21] In the present study, fasting plasma triglyceride levels were similar for both carbohydrates but

significantly higher for sucrose-fed rats in the postprandial state compared with cornstarch, which in turn may be related to the decrease of cholesterol and triglycerides observed in the liver. Previous work from Martineau and Deshaies[22] demonstrated that sucrose feeding increases triglyceride-rich lipoprotein secretion by the liver, mainly VLDL particles, thus decreasing their hepatic concentrations and increasing their levels in circulation. Plasma triglyceride levels may also be modified by their degradation. The main enzyme for triglyceride catabolism in plasma is lipoprotein lipase (LPL), which tends to be lower when rats are fed sucrose rather than cornstarch,[22] thus keeping triglycerides longer in circulation before they are broken down. Thus the high insulin response observed in the rats fed casein-sucrose in the present study could be associated with increased hepatic VLDL triglyceride secretion[23] and with a delayed removal of VLDL triglycerides due to insufficient induction of postheparin lipoprotein lipase activity,[24] inducing hypertriglyceridemia.

Fasted and postprandial cholesterol, HDL-C, and triglyceride levels were similar for cod and soy protein. Both proteins exerted a hypocholesterolemic effect as compared with casein, which is in accordance with the observations of Iritani et al.[7] Bergeron et al.[25] have shown in rabbits that cod protein increased postheparin lipoprotein lipase activity in the blood when compared with soy protein. Thus it is possible that in the present study, cod protein would have increased postheparin lipoprotein lipase activity in rats, which in turn would stimulate VLDL catabolism and lower triglyceride and cholesterol levels in plasma, resulting in a similar hypocholesterolemic effect to that observed with soy protein when compared with casein. The increase of HDL-C with casein compared with cod protein is not necessarily beneficial in rats since HDL is the predominant class of plasma lipoproteins.[26] Rat HDL-C is generally increased in hyperlipidemic conditions. In humans a different picture appears: casein diets tend to decrease plasma HDL-C,[27] increasing the risk for atherosclerosis.

The hypocholesterolemia produced by cod protein was not reflected by a decrease in liver lipids. Cod protein significantly increased hepatic cholesterol and triglycerides at a level similar to those with casein when compared with soy protein. It has already been shown that high insulin or low glucagon secretion in plasma produced increased hepatic triglycerides[28,29] and cholesterol[30] synthesis. Thus it seems reasonable to suggest that the higher insulin to glucagon ratio observed with cod protein as compared with soy protein in the postprandial state would lead to an increase of cholesterol and triglyceride synthesis in the liver. However, the low levels of glucose, cholesterol, and triglycerides in plasma suggest a normal glucose tolerance and insulin sensitivity in rats fed cod protein.

Consistent with prior observations made on soy protein versus casein[12] and cornstarch versus sucrose[31] separately, the combination of soy protein-cornstarch in the present study was shown to reduce the ability of this diet to induce insulin resistance as compared with the casein-sucrose diet. The postprandial insulin levels following the casein-sucrose diet, being significantly higher than those following the soy protein-cornstarch and soy protein-sucrose diets, may be attributed to the fact that casein[32] and sucrose[31] can separately stimulate insulin secretion and develop insulin resistance. As previously observed by Bergeron et al.,[25] no effect of cod protein versus soy protein on fasting plasma insulin levels was noted in this study. However, the higher postprandial insulin levels with the cod protein-cornstarch diet compared with the soy protein-cornstarch diet in the present study could be an explanation of the higher postheparin lipoprotein lipase activity observed by Bergeron et al.[25] in rabbits fed cod protein compared with soy protein in the presence of cornstarch, since insulin is known to stimulate LPL activity.[24] Moreover, the higher postprandial insulin levels with cod protein-cornstarch diet as compared with cod protein-sucrose and the other diets demonstrate that cod protein may reverse the carbohydrate effect usually observed when combined with casein in the regulation of plasma insulin levels in the rat. In that respect, amino acids and peptides released during protein digestion may influence insulin secretion through different mechanisms such as increased specific blood amino acids after protein digestion and stimulated release of gastrointestinal hormones following protein intake and the digestion process.[33] Interestingly, animal proteins such as cod protein contain high levels of essential amino acids including leucine[5] known to be secretagogue of insulin[34] but there is also a possibility that gastrointestinal peptides, which have been reported to stimulate insulin secretion,[35] may be specifically released during the digestion of cod protein. The different insulin responses induced after feeding cod protein and casein with either sucrose or cornstarch may suggest the hypothesis that the mechanisms involved in insulin secretion with either cod protein or casein are different and are mediated in a different manner by the source of carbohydrate present in the diet.

The present data also demonstrate that the dietary proteins and carbohydrates may influence fasting and postprandial lipid and glycemic metabolisms and that these effects may be mediated by a major hormonal mechanism such as the insulin to glucagon ratio and insulin sensitivity. Casein combined with sucrose in a low-fat high-carbohydrate diet increased plasma and hepatic cholesterol and triglycerides, and this effect may be mediated by the development of insulin resistance in these animals. In contrast, the high hepatic cholesterol and triglyceride levels observed with the cod protein-cornstarch diet may be related to a high postprandial insulin to glucagon ratio known to stimulate lipogenesis. The low total plasma cholesterol and triglycerides with this diet suggest a reduced efflux of lipids from the liver to the blood. The low fasting plasma glucose levels with cod protein-cornstarch diet indicate that the increase of postprandial insulin levels did not create an insulin resistance state. We therefore suggest that casein and cod protein modulate lipid and glucose metabolisms through insulin levels by two different mechanisms. Since the interaction of dietary proteins and carbohydrates induced different insulin levels after a 3-hr meal, it would be interesting to measure postprandial insulin levels at different times after these diets in order to determine a more complete insulin pattern. These results also emphasize the need to measure the levels of plasma amino acids and gastrointestinal hormones following cod protein and casein diets with either sucrose or cornstarch.

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References

1 Hamilton, R.M.G. and Carroll, K.K. (1976). Plasma cholesterol levels in rabbits fed low fat, low cholesterol diets. Effects of dietary proteins, carbohydrates and fibre from different sources. *Atherosclerosis* **24,** 44–62

2 Sugano, M., Ishiwaki, N., Nagata, Y., and Imaizumi, K. (1982). Effects of arginine and lysine addition to casein and soya-bean protein on serum lipids, apolipoproteins, insulin and glucagon in rats. *Brit. J. Nutr.* **48,** 211–221

3 Kritchevsky, D., Tepper, S.A., Czarnecki, S.K., and Klurfeld, D.M. (1982). Atherogenicity of animal and vegetable proteins. Influence of the lysine to arginine ratio. *Atherosclerosis* **41,** 429–431

4 Pfeuffer, M. and Barth, C.A. (1992). Dietary sucrose but not starch promotes protein-induced differences in rates of VLDL secretion and plasma lipid concentrations in the rat. *J. Nutr.* **122,** 1582–1586

5 Jacques, H., Deshaies, Y., and Savoie, L. (1986). Relationship between dietary proteins, their in vitro digestion products, and serum cholesterol in rats. *Atherosclerosis* **61,** 89–98

6 Bergeron, N. and Jacques, H. (1989). Influence of fish protein as compared to casein and soy protein on serum and liver lipids, and serum lipoprotein cholesterol levels in the rabbit. *Atherosclerosis* **78,** 113–121

7 Iritani, N., Narita, R., Fujita, T., and Tanaka, T. (1985). Effects of dietary fish protein, soybean protein and casein on cholesterol turnover in rats. *J. Nutr. Sci. Vitaminol.* **31,** 385–392

8 Cameron-Clarke, A. and Manchester, K.L. (1985). Effect of dietary carbohydrate and lipid on very low density lipoprotein secretion by rat hepatocytes. *Nutr. Rep. Int.* **32**(6), 1451–1460

9 Bernal, C., Basilico, M.Z., Gutman, R., and Lombardo, Y.B. (1989). Secretion and removal rates of very low density lipoprotein triglycerides at the three metabolic periods of hypertriglyceridemia induced by a sucrose rich diet. *Nutr. Rep. Int.* **40,** 71–83

10 Reaven, G.M., Risser, T.R., Chen, Y.-D., and Reaven, E.P. (1979). Characterisation of a model of dietary-induced hypertriglyceridemia in young, non-obese rats. *J. Lipid Res.* **20,** 371–378

11 Hubbard, R.W. and Sanchez, A. (1990). Dietary protein control of serum cholesterol by insulin and glucagon. In *Dietary proteins, cholesterol metabolism and atherosclerosis,* Vol. 16 *Monographs on Atherosclerosis* (M. Sugano, A.C. Beynen eds.), p. 139–147, Karger, Basel

12 Galibois, I., Jacques, H., Montminy, C., Bergeron, N., and Lavigne, C. (1992). Independent effects of protein and fibre sources on glucose and cholesterol metabolism in the rat. *Nutr. Res.* **12,** 643–655

13 Burnstein, M., Scholnick, H.R., and Morfin, R.V. (1970). Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. *J. Lipid Res.* **11,** 583–595

14 Folch, J., Lees, M., and Stanley, S. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226,** 497–508

15 Unger, R. and Elsentraut, A. (1968). Glucagon. In *Hormones in Blood,* Vol. 1, 2nd ed. (C. Gray, ed.), p. 83–128, Academic Press, New York, NY USA

16 Desbuquois, B. and Aurback, G.D. (1971). Use of polyethylene glycol to separate free and antibody bound peptide hormones in radioimmunoassays. *J. Clin Endocrinol. Metab.* **33,** 732–738

17 Keppler, D. and Decker, K. (1974). Glycogen determination with amyloglucosidase. In *Methods of Enzymatic Analysis,* 2nd ed. (M.V. Bergmeyer, ed.), p. 1127–1131, Academic Press, New York, NY USA

18 Montgomery, D.C. (1984). *Design and Analysis of Experiments,* 2nd ed., p. 189–246, John Wiley & Sons, New York, NY USA

19 Beynen, A.C. (1990). Comparison of the mechanisms proposed to explain the hypocholesterolemic effect of soybean versus casein in experimental animals. *J. Nutr. Sci. Vitaminol.* **35**(Suppl.), S87–S93

20 Storlien, L.H., Kraegen, E.W., Jenkins, A.B., and Chisholm, D. J. (1988). Effects of sucrose vs starch diets on in vivo insulin action, thermogenesis, and obesity in rats. *Am. J. Clin. Nutr.* **47,** 420–427

21 Reaven, G.M. (1988). Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes* **37,** 1595–1607

22 Martineau, M.-J. and Deshaies, Y. (1990). Fasting and postprandial changes in determinants of triacylglycerol metabolism in rats given meals high in starch or sucrose. *Nutr. Res.* **10,** 1215–1226

23 Topping, D.L. and Mayes, P.A. (1976). Comparative effects of fructose and glucose on the lipid and carbohydrate metabolism of perfused rat liver. *Brit. J. Nutr.* **36,** 113–126

24 Cryer, A. (1981). Tissue lipoprotein lipase activity and its action in lipoprotein metabolism. *Int. J. Biochem.* **13,** 525–541

25 Bergeron, N., Deshaies, Y., and Jacques, H. (1992). Dietary fish protein modulates high density lipoprotein cholesterol and lipoprotein lipase activity in rats. *J. Nutr.* **122,** 1731–1737

26 Chapman, M.J. (1986). Comparative analysis of mammalian plasma lipoproteins. In *Methods of Enzymology,* Vol. 128, Part A (J.P. Segrest and J.J. Albers, eds.), p. 70–143, Academic Press, Montreal, Canada

27 Meinertz, H., Nilaussen, K., and Faergeman, O. (1989). Soy protein and casein in cholesterol-enriched diets: effects on plasma lipoproteins in normolipidemic subjects. *Am. J. Clin. Nutr.* **50,** 786–793

28 Reaven, G.M., Lerner, R.L., Stern, M.P., and Farquhar, J.W. (1967). Role of insulin in endogenous hypertriglyceridemia. *J. Clin. Invest.* **46,** 1756–1767

29 Schade, D.S., Woodside, W., and Eaton, R.P. (1979). The role of glucagon in the regulation of plasma lipids. *Metabolism* **28,** 874–886

30 Dugan, R.E., Ness, G.C., Lakshmann, M.R., Nepokroeff, C.M., and Porter, J.W. (1974). Regulation of hepatic β-hydroxy-β-methylglutaryl coenzyme A reductase by the interplay of hormones. *Arch. Biochem. Biophys.* **161,** 499–504

31 Hallfrisch, J., Cohen, L., and Reiser, S. (1981). Effect of feeding rats sucrose in a high fat diet. *J. Nutr.* **111,** 531–536

32 Baba, N., Radwan, H., and Van Itallie, T. (1992). Effects of casein versus soy protein on body composition and serum lipid levels in adult rats. *Nutr. Res.* **12,** 279–288

33 Milner, R.D.G. (1970). The stimulation of insulin release by essential amino acids from rabbit pancreas in vitro. *J. Endocrinol.* **47,** 347–352

34 Reaven, G. and Grecaberg, R.E. (1965). Experimental leucine-induced hypoglycemia in mice. *Metabolism* **14,** 625–631

35 Thomas, F.B., Mazzaferri, E.L., Crockett, S.E., Makhjian, H.S., Gruemer, H.D., and Cataland, S. (1976). Stimulation of secretion of gastric inhibitory polypeptide and insulin by intraduodenal amino acid perfusion. *Gastroenterology* **70,** 523–527