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Metabolic Adaptation to a Low Carbohydrate-High Protein ('Traditional') Diet in Australian Aborigines

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Summary. We have investigated plasma glucose and insulin responses to 75 g glucose in 12 young, full-blood Aborigines before and after 2 weeks on a diet derived almost exclusively from seafood. This diet was low in fat, extremely low in carbohydrate and high in protein and was representative of the diet consumed by these people in their traditional lifestyle during those times of the year when very little vegetable food was available.

After an initial weight loss which was probably due to glycogen, salt and water losses associated with the dietary change, body weights stabilised by the end of the first week. Total triglyceride concentrations in fasting plasma fell from 1.32 ± 0.33 before the diet to 0.61 ± 0.08 mmol/l after it, while total cholesterol, which was low initially, did not fall significantly.

There was a small but significant improvement in glucose tolerance and a small reduction in insulin response indicating that the Aborigines had adapted effectively to the very low carbohydrate-high protein diet in the 2 week period. The insulin response to 50 g protein also fell significantly after the seafood diet. The results suggest that glucose tolerance is not determined solely by the carbohydrate content of the diet, but rather by the availability of carbohydrate either directly or indirectly in precursor form as dietary protein.

Key words: Australian Aborigines, diabetes, insulin secretion, glucose tolerance, low carbohydrate-high protein diet, triglycerides.

High prevalence rates for Type 2 (non-insulin-dependent) diabetes mellitus have been reported for Australian Aborigines when they urbanize [1–5]. It has been suggested that metabolic characteristics which favoured survival in the traditional hunter-gatherer lifestyle (the 'thrifty gene' [6]) became detrimental as traditional diet and physical activity patterns were abandoned. Consistent with a metabolism favouring efficient fat deposition, several studies have reported high insulin responses to an oral glucose challenge in Aborigines [2, 7, 8]. We have shown that although this unusually high insulin response was reduced by temporary reversion to traditional lifestyle, it was still significantly higher than in age- and weight-matched Caucasoids [7].

Traditional lifestyle was characterized by a high level of physical activity and a diet derived from a wide variety of animal and vegetable sources. In certain areas of Australia there were periods each year when very little vegetable food was available and consequently the diet was extremely low in carbohydrate. Although we have previously shown that glucose tolerance was not impaired by three months on a low carbohydrate-high protein diet [7], we have no information on short term adaptation to such a diet. We approached this question in the present study by measuring glucose tolerance and insulin secretion before and after 2 weeks on a diet derived exclusively from seafood (i.e. very low carbohydrate-high protein diet). We also measured the insulin response to an oral protein load to see if it was affected by the high protein diet.

Methods

Subjects

Twelve full-blood Aborigines (ten women, two men) from the Mowanjum Community, Derby, Western Australia, participated in this study. Their mean age was 24.3 ± 1.0 years, mean body weight 66.8 ± 3.7 kg and mean body mass index 23.6 ± 1.4 kg/m^2 (Table 1). They were weight stable before the study and there were no known diabetic subjects in the group.

Field Study

The field study was carried out in an isolated location on the north-west coast of Australia 250 km north-east of Broome (latitude 17°). The Aborigines had no access to store foods or beverages during the

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Table 1. The changes in body weight and fasting plasma triglyceride and cholesterol concentrations in 12 Aborigines after 2 weeks on a seafood diet

Subjects	1	2	3	4	5	6	7	8	9	10	11	12	Mean ± SEM
Sex	F	F	F	F	F	F	F	F	F	F	M	M	
Age (years)	25	21	23	27	28	24	26	28	27	17	21	25	24 ±1
Height (cm)	159	163	174	161	170	166	164	166	169	173	181	174	168 ±2
Body mass index (kg/m^2)	25.3	18.8	22.5	18.1	24.6	22.5	36.1	23.6	26.6	22.7	18.9	23.5	23.6 ±1.4
Weight (kg)													
baseline	64	50	68	47	71	62	97	65	76	68	62	71	66.8 ±3.7
1 week	63	48	66	46	67	60	93	61	74	66	60	69	64.4 ±3.5
2 weeks	61	50	64	47	68	58	93	62	72	64	61	70	64.1 ±3.3
Plasma triglyceride (mmol/l)													
baseline	0.79	0.68	2.23	0.97	0.99	1.15	1.22	3.48	1.29	0.90	1.15	1.10	1.33 ± 0.23
2 weeks	0.41	0.54	0.59	0.43	0.90	0.66	1.24	0.84	0.56	0.52	0.34	0.34	0.61 ± 0.08
Plasma cholesterol (mmol/l)													
baseline	3.9	4.1	5.6	2.6	5.2	3.8	2.9	5.2	4.0	3.3	6.0	7.0	4.3 ±0.6
2 weeks	2.8	3.6	3.8	3.7	4.6	3.4	2.8	5.6	4.6	2.4	5.8	4.1	3.9 ±0.3

study. One of us (K. O.'D.) was present throughout the study to ensure strict compliance to the experimental diet. The participants travelled from Derby by car and had at least one full day without alcohol before the metabolic tests were performed. The seafood diet was then commenced and 2 weeks later the baseline studies were repeated. The food gathering component of this study did not involve high energy expenditure on the part of the participants since most of the food eaten came from fish caught in nets on the incoming and outgoing tides.

Urban Diet

The main dietary components were unenriched white flour, white sugar, white rice, carbonated drinks, alcoholic beverages (beer, port), powdered milk and cheap fatty meat. Other than onions and potatoes very little fresh vegetables or fruits were consumed. This diet was high in refined carbohydrate (40–50%) and fat (40–50%) and relatively low in protein ($\leqq$ 10%).

Experimental Diet

This diet was derived almost exclusively from seafood. The main source of food was a variety of fish (mullet, whiting, blue salmon, trevally, skippy, catfish) supplemented with shellfish (oysters, mudcrabs, snails, cockles). Small and medium sized fish, crabs, snails etc. were grilled whole on the coals from wood fires, while large fish were sometimes cut up and used for soup. Every 2 or 3 days a handful of fibrous berries were eaten as a prophylaxis against constipation. Samples of seafood eaten were taken for analysis and preliminary results indicate a diet of the following composition: protein 70–75%, fat 20–25%, carbohydrate < 5%. These analyses were made on raw frozen seafood. The loss of some fat during cooking (grilling etc) cannot be excluded.

Metabolic Studies

On the 2 days immediately before commencing the experimental diet, the subjects underwent two metabolic tests after a 12 h overnight fast. An indwelling IV cannula was inserted into a vein in the forearm and kept patent with heparinized saline. A fasting blood sample was taken before the subjects were given either 75 g glucose ('Glucola') or 50 g protein (250 g veal: 19.8% protein, 1.3% fat) on consecutive days. The meat was consumed over 5–10 min and zero time was taken as the commencement of eating. Post-prandial blood samples were taken at ½, 1, 2 and 4 h. These studies were repeated after 2 weeks on the experimental diet.

Blood samples were centrifuged immediately and the separated plasma stored frozen until flown to Melbourne for analysis of plasma glucose, insulin and lipids.

Analytical Methods

Glucose concentrations were measured in fluoride oxalate plasma by the glucose oxidase method. Immunoreactive insulin concentrations in heparinized plasma were measured using dextran-coated charcoal for precipitation of free hormone after reaction of insulin with commercially available antiserum (Burroughs-Wellcome). Human insulin (Novo) was used as the standard. The range of the insulin assay was 5–200 mU/l and the interassay coefficient of variation was 8%. Fasting triglyceride concentrations were determined enzymatically after enzymatic hydrolysis using a Technicon autoanalyser. The normal range for triglyceride concentrations in fasting plasma of Caucasoid Australians is 0.5–2 mmol/l. Total cholesterol concentration in fasting plasma was measured colorimetrically after reaction with acetic anhydride and concentrated sulphuric acid using a commercially available kit (Boehringer). The normal range for cholesterol concentration in fasting plasma from Caucasoids is 3.5–6.5 mmol/l.

Statistical Methods

Statistical analysis of the results was by multiple regression analysis, analysis of variance and Student's paired t-tests.

Results

The changes in body weights of the subjects in response to the experimental diet are reported in Table 1. Weight loss over the 2 week period was variable (0–4 kg) and much more pronounced during the first week of the diet (mean 2.4 kg) than the second (mean 0.3 kg). The four leanest subjects lost the least weight.

Fasting plasma cholesterol concentrations were not significantly affected by the dietary change (Table 1). In contrast, fasting plasma triglyceride concentrations fell

Table 2. The changes in plasma glucose concentrations in 12 Aborigines following 75 g oral glucose before and after 2 weeks on a seafood diet

Subjects		1	2	3	4	5	6	7	8	9	10	11	12	Mean ± SEM
Sex		F	F	F	F	F	F	F	F	F	F	M	M	
Age (years)		25	21	23	27	28	24	26	28	27	17	21	25	24 ±1
Height (cm)		159	163	174	161	170	166	164	166	169	173	181	174	168 ±2
Body mass index (kg/m^2)		25.3	18.8	22.5	18.1	24.6	22.5	36.1	23.6	26.6	22.7	18.9	23.5	23.6±1.4
Plasma glucose (mmol/l)														
baseline	0 h	7.5	4.4	5.1	4.9	5.3	5.2	4.1	4.5	4.9	5.2	5.0	4.7	5.1±0.2
	½ h	10.8	7.4	7.8	6.2	9.4	6.6	5.6	7.9	8.3	6.8	6.9	7.3	7.6±0.4
	1 h	14.6	8.2	8.0	7.1	10.6	5.3	6.5	10.5	8.2	6.2	7.3	7.9	8.4±0.7
	2 h	11.7	7.8	7.2	6.4	8.4	4.9	4.8	8.6	5.2	6.2	6.6	6.7	7.0±0.6
	4 h	8.8	3.3	7.7	4.0	4.1	4.6	4.1	4.7	4.6	–	4.1	4.1	5.1±0.5
2 weeks	0 h	5.6	5.1	4.3	5.3	5.1	4.4	4.4	4.8	4.5	5.2	4.5	4.9	4.8±0.1
	½ h	8.5	7.4	5.0	7.2	7.7	8.5	5.2	8.3	6.2	6.7	7.3	7.2	7.1±0.3
	1 h	9.4	8.5	9.1	7.4	9.4	8.9	5.3	8.9	6.7	8.3	7.0	6.9	8.0±0.4
	2 h	8.8	6.8	10.4	4.6	5.8	7.2	4.4	5.7	4.7	7.7	4.3	6.0	6.4±0.5
	4 h	5.9	4.2	7.7	5.7	5.2	3.2	3.8	4.0	4.1	4.5	4.3	4.0	4.7±0.4
Area under glucose curve ($mmol \cdot l^{-1} \cdot h^{-1}$)														
baseline		44.6	26.2	29.7	23.3	30.2	20.5	20.0	30.6	23.9	25.2	24.2	24.9	26.9±1.9
2 weeks		29.0	21.9	32.5	23.1	26.1	26.0	18.1	24.6	20.4	26.9	20.7	23.0	24.4±1.2

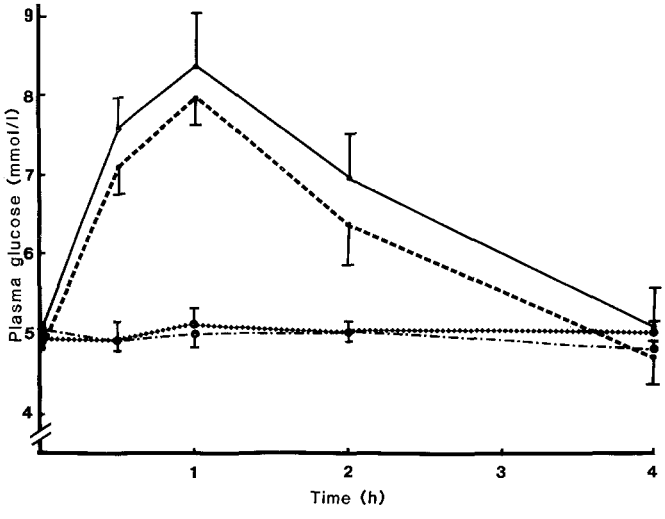

Fig. 1. Change in plasma glucose concentrations in response to 75 g glucose or 50 g protein in 12 Aborigines before (— glucose, ◆◆◆◆◆ protein) and after (- - - - glucose, -·-·- protein) 2 weeks on a seafood diet. Results expressed as mean ± SEM

to less than half the initial levels after 2 weeks on the seafood diet ($p < 0.001$).

The change in plasma glucose in response to the glucose load before and after the seafood diet is shown in Table 2 and Figure 1. Three way analysis of variance of these data revealed a small but significant improvement in glucose tolerance in response to the very low carbohydrate diet ($p < 0.05$). Multiple regression analysis indicated that there was no relationship between improvement in glucose tolerance and weight loss. Plasma glucose did not change after the ingestion of the protein load (Fig. 1) either before or after the seafood diet.

The insulin responses to oral glucose before and after the seafood diet are shown in Table 3 and Figure 2. Although peak insulin concentrations in response to oral glucose were not reduced by the diet, three way analysis of variance indicated that the insulin response over the 4-h test period was reduced by the seafood diet ($p < 0.05$). However, multiple regression analysis indicated that the reduction in insulin response was not related to weight loss. The insulin response to the protein load (which was much smaller than to the glucose load) was also significantly reduced after 2 weeks on the experimental diet ($p < 0.01$).

Discussion

The major finding in the present study was the lack of deterioration of glucose tolerance in response to 2 weeks on a diet which was extremely low in carbohydrate. Indeed, the Aborigines in this study showed a small but significant improvement in glucose tolerance which was accompanied by a similar small reduction in insulin response. Together these findings suggest an improvement in glucose utilization and insulin sensitivity after the high protein-low carbohydrate diet. These changes were not correlated with weight loss in the individual subjects. The weight loss occurred primarily in the first week of the seafood diet and was probably due in large part to the major changes in salt and water balance which occur on low carbohydrate diets [9, 10].

Furthermore, it is unlikely that these changes were due to alcohol withdrawal. In a previous study [8] we found no differences in glucose tolerance or insulin responses between two groups of Aborigines closely

Table 3. The changes in plasma insulin concentrations in 12 Aborigines following 75 g oral glucose before and after 2 weeks on a seafood diet

Subjects		1	2	3	4	5	6	7	8	9	10	11	12	Mean ± SEM
Sex		F	F	F	F	F	F	F	F	F	F	M	M	
Age (years)		25	21	23	27	28	24	26	28	27	17	21	25	24 ± 1
Height (cm)		159	163	174	161	170	166	164	166	169	173	181	174	168 ± 2
Body mass index (kg/m^2)		25.3	18.8	22.5	18.1	24.6	22.5	36.1	23.6	26.6	22.7	18.9	23.5	23.6 ± 1.4
Plasma insulin (mU/l)														
baseline	0 h	29	3	12	5	7	12	8	12	18	22	2	2	11 ± 2
	½ h	68	36	44	26	33	109	56	80	84	88	49	29	59 ± 8
	1 h	95	60	51	40	42	33	63	97	110	63	58	38	63 ± 7
	2 h	107	67	48	64	37	41	23	99	46	92	39	34	58 ± 8
	4 h	48	10	7	7	5	7	8	35	13	–	2	2	13 ± 4
2 weeks	0 h	11	11	9	12	8	6	23	4	10	14	5	2	10 ± 2
	½ h	34	41	46	32	35	73	54	49	61	34	48	24	44 ± 4
	1 h	45	66	70	61	51	103	66	76	80	46	59	34	63 ± 5
	2 h	65	69	60	34	21	51	31	42	46	39	19	17	41 ± 5
	4 h	36	28	6	33	11	7	5	3	5	17	6	2	13 ± 4
Area under insulin curve ($mU \cdot l^{-1} \cdot h^{-1}$)														
baseline		321	174	142	134	110	147	120	299	161	252	129	97	174 ± 22
2 weeks		200	209	175	149	100	199	134	149	167	167	104	66	152 ± 13
Area under insulin curve														
Area under glucose curve (mU/mmol)														
baseline		7.3	6.6	4.8	5.8	3.7	7.2	6.0	9.8	6.7	10.0	5.3	3.9	6.4 ± 0.6
2 weeks		6.9	9.5	5.4	6.5	3.8	7.7	7.4	6.1	8.2	6.2	5.0	2.9	6.3 ± 0.5

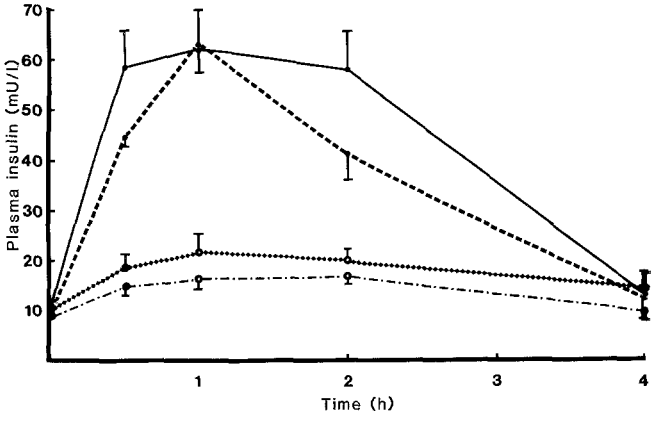

Fig. 2. Change in plasma insulin concentrations in response to 75 g glucose or 50 g protein in 12 Aborigines before (— glucose, ◆◆◆◆◆ protein) and after (- - - - glucose, -·-·- protein) 2 weeks on a seafood diet. Results expressed as mean ± SEM

matched for age and body weight but with quite different alcohol intakes: the 'rural' group consumed no alcohol, while the 'urban' group were generally heavy drinkers having abstained for at least one day prior to testing (as in the present study). Nor is it likely that the improvement in glucose tolerance and reduction in insulin response were due to increased physical activity since procuring the experimental diet did not involve high energy expenditure on the part of the participants. Most of the food eaten came from fish caught in nets on the incoming and outgoing tides.

Composition of the diet plays an important role in the pathogenesis and therapy of Type 2 diabetes. Epidemiological studies indicate that Type 2 diabetes prevalence in a population is directly related to the energy and fat consumption and inversely related to the carbohydrate content of the diet [11, 12]. Consistent with this, glucose tolerance has been shown to be impaired by low carbohydrate-high fat diets [13–15] and improved by high carbohydrate diets [13, 14, 16, 17]. As a result, it is now widely accepted that, as originally proposed by Himsworth [13], glucose tolerance is determined solely by the carbohydrate content of the diet. However there are few data available on the effect of high protein-low carbohydrate diets on glucose tolerance, which is the essence of the diet studied here. One of the only other published studies of the effects on glucose tolerance was that of Heinbecker [18] on Eskimos in which he found normal glucose tolerance on a high protein-low carbohydrate diet. These results in Eskimos suggest that our observations in Australian Aborigines are not racially determined.

The mechanisms by which low carbohydrate diets which are high in protein preserve glucose tolerance while those high in fat do not, is probably related to the gluconeogenic potential of high protein diets. Elevated glucose levels in response to the ingestion of protein would promote hepatic gluconeogenesis from amino acids entering the liver from the splanchnic circulation. In this way a low carbohydrate diet which was high in protein could maintain the necessary glucose supply to the body whereas one high in fat could not.

The striking fall in fasting plasma triglyceride concentration after the seafood diet was probably due to a

combination of the high content of $\omega 3$ polyunsaturated fatty acids [19, 20] and the low content of carbohydrate and abstinence from alcohol in the experimental diet [7, 8]. Only two of 12 of the baseline triglyceride concentrations were over 2 mmol/l (2.23, 3.48 mmol/l) and they both fell dramatically (0.59, 0.84 mmol/l respectively). The other 10 values ranged from 0.8 to 1.29 mmol/l before the diet and 0.34 to 1.24 mmol/l after it. Triglyceride concentrations fell in all but one subject. Undoubtedly withdrawal of alcohol contributed to the marked fall in the two subjects who were high initially and may have contributed to a small extent in the others. However, falls of this magnitude have been reported in studies of fish feeding in Caucasoid subjects where alcohol was not a complicating factor [19, 20]. Fasting plasma cholesterol concentrations were similar to levels reported in other studies with people from this area [7, 8]. That they did not change may have been due to the high content of polyunsaturated fatty acids in seafood.

The mild impairment of glucose tolerance and high insulin response to oral glucose observed in this group of Aborigines are very similar to data obtained from other Aborigines from the same area [8]. These metabolic characteristics may have been important to survival in the traditional lifestyle by favouring efficient fat deposition – the 'thrifty gene' [6] – on a diet in which the carbohydrate content was seasonally variable. As the Aborigines change from the traditional to the urban diet which is high in refined carbohydrate, it is possible that these metabolic characteristics become detrimental and facilitate the development of obesity and diabetes.

Type 2 diabetes has been an increasing health problem in Aboriginal communities as they make the transition from traditional to urban lifestyle and conventional therapies have failed for a variety of cultural, historic and economic reasons. Adopting elements of the traditional lifestyle periodically would provide a practical and acceptable approach to the problem.

In conclusion, the present study has demonstrated that glucose tolerance is not determined solely by the amount of carbohydrate in the diet, but rather by the availability of carbohydrate either directly in the diet or in the precursor form as dietary protein. These results, together with those from a previous study of longer duration [7], suggest that such a diet may have an important role in the primary prevention of diabetes in Australian Aborigines.

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