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Diet- and diabetes-induced change in insulin binding to the nuclear membrane in spontaneously diabetic rats is associated with change in the fatty acid composition of phosphatidylinositol

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Abstract

Insulin binding to the nucleus in vivo alters the binding of transcription factors to the promoter region of lipogenic genes, thereby changing expression of these genes. The present research was designed to investigate whether change in diet fat composition alters insulin binding to nuclear insulin receptors at various stages of onset of diabetes in spontaneously diabetic B/B rats. The fatty acid composition of lipids comprising the nuclear membrane was also examined. Weanling rats were fed a nonpurified diet (low-fat commercial rat chow) or a semipurified diet containing 20 g/100 g fat of either high (1.0) or low (0.25) polyunsaturated to saturated (P/S) fatty acid ratio. Insulin binding to liver nuclei was measured when the blood glucose level was 100 mg/dl and 400 mg/dl. No effect of diet treatment on age of onset of diabetes was found. Specific binding of insulin to nuclei from rats with a blood glucose level of 100 mg/dl did not differ from nondiabetic rats, and was higher than in diabetic rats with a blood glucose level of 400 mg/dl. Insulin binding was greater in rats fed a high P/S diet. The high versus low P/S diet treatment primarily altered the fatty acid composition of phosphatidylinositol in the nuclear membrane. Diabetic rats fed nonpurified diet showed a significant increase in levels of 18:2(n-6) and 22:6(n-3), whereas 20:4(n-6) decreased in the phosphatidylcholine fraction compared with control rats fed chow. As rats became diabetic, the level of monounsaturated fatty acids, 18:2(n-6) and 22:6(n-3) decreased, whereas the level of 20:4(n-6) increased in phosphatidylinositol. Change in the composition of these nuclear membrane components may be associated with transitions in insulin binding.

Keywords: Diet fat; Diabetes; Insulin; Nucleus; Rats

1. Introduction

The B/B rat exhibits many features of human Type I diabetes [1,2]. Diabetes in spontaneously diabetic B/B rats results from an autoimmune disease where insulin-producing β-cells of the pancreas are destroyed. Initially, β-cell and pancreatic insulin concentration is normal in B/B rats; however, when B/B rats become diabetic, immunoreactive insulin declines to almost zero [3,4]. The characteristic reduction in insulin concentration associated with diabetes mellitus increases insulin receptor sites and binding capacity for insulin in some target tissues [5–8].

Previous studies have shown that feeding a high-polyunsaturated-fat diet alters plasma membrane composition and increases insulin binding and insulin-stimulated functions in adipocytes from control and diabetic rats [6] and IGF-1 binding in muscle [9]. Insulin binding sites have also been reported on nuclear membrane [10,11] and in vivo binding to the nuclear envelope increases in response to a glucose meal [12]. This binding of insulin to the nucleus results in signal transduction by change in the phosphorylation state of transcription factors that bind to the promoter region of lipogenic genes, resulting in enhanced expression of these genes in response to a carbohydrate meal [13]. We have also shown that feeding diets differing in fatty acid composition alters liver nuclear membrane composition and insulin binding to liver nuclei in lean and obese mice [14]. Diets differing in amount and type of fat also alter the outcome of several autoimmune diseases [1,15–17].

This study was designed to determine whether diet fat

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composition alters specific binding of insulin to liver nuclei of spontaneously diabetic B/B rats at early and late stages of onset of diabetes. Control and diabetic rats were compared to assess whether increased consumption of polyunsaturated fatty acids normalize the altered composition of membrane phospholipids expected in the diabetic state and whether the fatty acid composition of membrane phospholipid that is important in signaling pathways is altered during change in this disease state.

2. Materials and methods

2.2. Rats and diets

Control and spontaneously diabetic B/B rats originally derived from the Wistar-Furth strain were obtained from Bio-Breeding Laboratories of Canada, Ltd. (Ottawa, Canada). The incidence of diabetes in B/B rats is between 40–70% and onset occurs between 60 and 120 days of age. Control and spontaneously diabetic B/B male weanling rats were randomly divided into three groups (eight rats per group). Extra rats were maintained in the diabetic groups to provide eight rats per treatment at each time point. One group received nonpurified diet (Purina chow) and the other two groups were fed semipurified diets containing 20% (w/w) fat of either low (0.25) or high (1.0) polyunsaturated to saturated (P/S) fatty acid ratio [6]. These diet comparisons represent the physiological range of dietary fatty acid composition consumed by humans. Rats were housed in individual wire-bottom cages at 23°C in a 12-h light/dark cycle, given ad libitum access to food and water and were weighed twice weekly. Blood glucose level was checked on alternate days and diabetes was diagnosed on the basis of a plasma glucose level in excess of 250 mg/dl, polyuria and failure to gain weight. In the spontaneously diabetic group, rats that attained a blood glucose level of 100 mg/dl were considered to be at the stage of onset of diabetes. Serum insulin was determined by radioimmune assay (Insulin RIA 100 kit, Pharmacia Co., Uppsala, Sweden) against a rat insulin standard.

2.3. Preparation of rat liver nuclei

Liver nuclei were isolated by modifications to the procedure of Widnell and Tata [18]. Liver homogenate in 0.32 M sucrose, 3 mM $MgCl_2$ was centrifuged at 3840 $\times$ *g* for 20 min. The crude nuclear pellet was resuspended in 2.4 M sucrose, 1 M $MgCl_2$ (pH 7.4) and centrifuged at 50,000 $\times$ *g* for 60 min. Purified nuclear pellet was resuspended in 0.25 M sucrose containing 50 mM Tris buffer and 3 mM $MgCl_2$ at pH 7.5 and centrifuged at 1090 $\times$ *g* for 10 min. The pellet was resuspended in a small volume of the same buffer. Marker enzymes (cytochrome oxidase, cytochrome c-oxidoreductase, glucose 6-phosphatase, glucose 6-phosphate dehydrogenase and 5′-nucleotidase) were measured to assess purity of the nuclei prepared [19]. The nuclei prepared showed less than 1% impurities from mitochondria, microsomes and plasma membrane. Micrographs of nuclear preparations were prepared for visual confirmation that nuclear preparations did not contain mitochondrial or plasma membrane contaminants.

2.4. Insulin binding assay

Insulin binding to freshly isolated nuclei was measured by incubating 200 μg of nuclear protein with 0.2 μg/ml of (^{125}I)-insulin (New England Nuclear, Dupont, Mississauga, Canada) and varying concentrations of unlabeled insulin in a total volume of 0.25 ml. Conditions for insulin binding were optimized. Incubations were performed at 25°C for 90 min. The reaction was stopped by adding 1.0 ml chilled assay buffer (0.25 M sucrose, 10 mM $MgCl_2$, 20 mM Tris-HCl buffer, pH 7.5, 2 mM Na_2 ethylenediaminetetraacetic acid, 5 mg/ml bovine serum albumin) and centrifuged at 12,000 rpm in a microfuge. The supernatant was discarded, pellets were washed a second time and counted in a Packard Bell gamma counter.

2.5. Extraction and analysis of nuclear lipids

Lipids were extracted from liver nuclei [20], and phospholipids were separated on silica gel H plates using the solvent system chloroform:methanol:propan-2-ol:0.25% (w/v) KCl: triethylamine (30:9:25:6:18 by volume) [21]. Separated phospholipids were sprayed with 0.1% (w/v) 8-anilino-1-naphthalene sulfonic acid and viewed under ultraviolet light. Phospholipid spots were scraped directly into tubes, fatty acid methyl esters prepared with 14% (w/v) BF_3 methanol reagent and separated by automated gas–liquid chromatography as described previously [22]. The analytical conditions separated all saturated, mono-, di- and polyunsaturated fatty acids from C14 to C24 in chain length.

2.6. Statistical analysis

The effect of diet treatment and physiological state on membrane phospholipid fatty acid composition and insulin binding was compared by two-way analysis of variance procedures. A Duncan's multiple range test was used to discriminate significant differences between individual treatment groups [23]. Scatchard analysis was done by plotting bound/free insulin versus bound insulin using nonlinear regression analysis.

Table 1
Effect of diet and diabetes on the body weight, liver weight and serum insulin of control and spontaneously diabetic B/B rats

Group diet	Blood glucose (mmol/l)	Final body weight (g)	Final liver weight (g)	Serum insulin (pmol/l)
Control				
Reference	84	374 ± 6.8	15.0 ± 0.9	88.3 ± 3.0
P/S = 1.0	84	404 ± 12.0[a]	14.2 ± 0.7[a]	82.0 ± 8.0[a]
P/S = 0.25	84	384 ± 11.0[a]	14.0 ± 1.2[a]	75.0 ± 6.0[a]
Spontaneously diabetic				
Reference	100	266 ± 1.8	10.8 ± 0.3	66.0 ± 5.0
P/S = 1.0	100	303 ± 7.3[b]	11.1 ± 0.2[b]	76.0 ± 9.4[a]
P/S = 0.25	100	311 ± 12.0[b]	11.0 ± 0.6[b]	68.0 ± 8.5[a]
Spontaneously diabetic				
Reference	400	240 ± 10.0	6.3 ± 0.4	30.0 ± 4.0
P/S = 1.0	400	311 ± 14.0[b]	9.1 ± 0.4[b]	26.0 ± 6.5[b]
P/S = 0.25	400	300 ± 12.0[b]	10.5 ± 1.4[b]	30.0 ± 3.4[b]

Weanling rats were fed either chow or semipurified diet high or low in polyunsaturated fatty acids as explained in methods. At 50 days postpartum, blood glucose level was checked on alternate days. Values are group means ± S.E., $n = 10$. Means in a column without a common superscript are different, $P < .05$.

3. Results

Spontaneously diabetic B/B rats weighed less and exhibited a lower serum insulin level than control animals (Table 1). Serum insulin level decreased further in B/B diabetic rats with increased blood glucose level. Diet fat composition did not affect these variables in control or B/B diabetic rats. The diet treatments did not influence the incidence of diabetes observed. For each diet treatment, animals became diabetic between 80–90 days after birth. These findings contrast those of Scott et al. [4] where feeding a defined diet with casein as the only protein source resulted in prevention of diabetes in B/B rats (0% incidence compared with 28% in chow-fed animals). For our studies, the weanling spontaneously diabetic rats were obtained from BioBreeding Labs (Ottawa, Canada), and prior to receiving the animals in our laboratory, the young rats had access to rat chow. The lack of effect of diet treatment on the onset of diabetes is now believed to be due to the presence of factors in rat chow, which exert their effect at a young age [24] to stimulate the autoimmune response. Other studies have also shown no influence of diets high in carbohydrate, protein or fat on diabetes incidence in B/B rats [24–27].

3.1. Insulin binding

Insulin binding to liver nuclei from B/B diabetic rats at a blood glucose level of 100 mg/dl was not different from control animals at an insulin concentration of 800 ng/ml for animals fed either of the diet treatments (Fig. 1). In the more-diabetic rats (blood glucose level of 400 mg/dl), insulin binding decreased. Rats fed the high P/S diet increased specific binding of insulin to nuclei of both control and B/B diabetic rats compared with rats fed the low P/S diet (Fig. 1). However, this increase was not large enough to restore insulin binding in diabetic animals to that of control rats.

Decreased binding appears to be due to a reduction in number of insulin binding receptors for diabetic B/B rats (blood glucose level of 400 mg/dl) fed all diet treatments. Only one class of binding sites was observed for nuclei from diabetic rats (data not shown). Scatchard analysis of insulin binding to nuclei from control rats fed the high P/S diet suggested greater binding due to a greater number of available binding sites compared with diabetic rats at a blood glucose level of 400 mg/dl. Definitive analysis of binding characteristics would require analysis using a wider range of conditions to enable determination of specific binding when the specific binding is saturated.

3.2. Phospholipid fatty acid composition

Fatty acid composition of phosphatidylcholine and phosphatidylinositol of liver nuclei was significantly altered by diet and by diabetes (Table 2). Major effects of disease state or diet treatment were not observed in phosphatidylethanolamine or phosphatidylserine; thus, data for these phospholipids is not illustrated.

3.2.1. Phosphatidylcholine

The phosphatidylcholine fraction in diabetic B/B rats (blood glucose level of 400 mg/dl) fed chow showed significant increase in 18:2(n-6) and 22:6(n-3) compared with control animals fed chow. The level of 20:4(n-6) was lower in chow-fed diabetic rats. Feeding a high P/S or low P/S diet did not alter the essential fatty acid content of phosphatidylcholine.

3.2.2. Phosphatidylinositol

In the phosphatidylinositol fraction, the level of monounsaturated fatty acids, 18:2(n-6) and 22:6(n-3) decreased

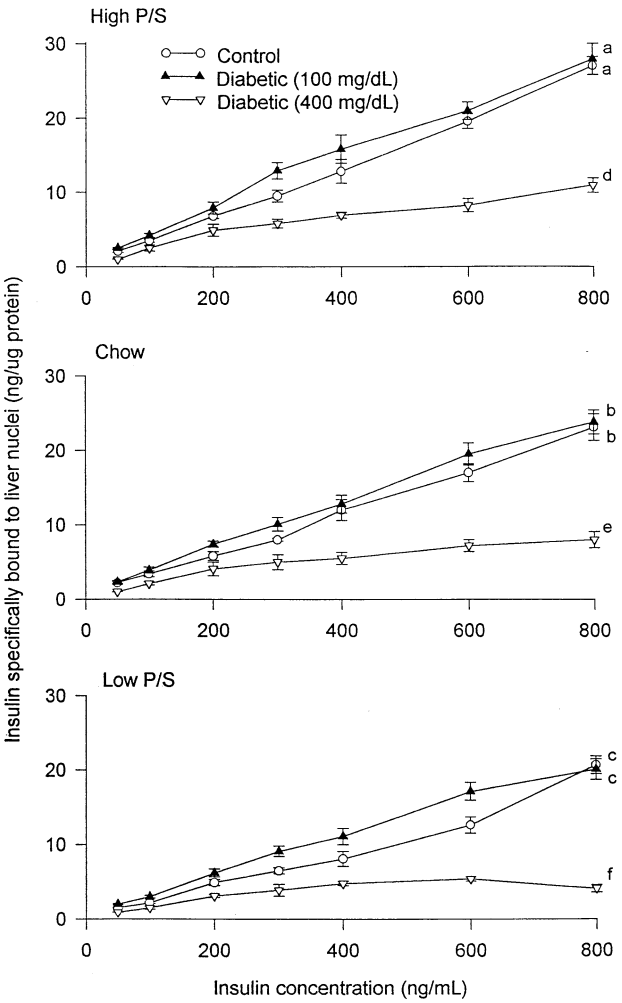

Figure 1. Total specific binding of insulin to liver nuclei of control and spontaneously diabetic B/B rats at 25°C: total specific bound insulin to liver nuclei from control animals (○), diabetic B/B animals at a blood glucose level of 100 mg/dl (▲) and diabetic B/B animals at a blood glucose level of 400 mg/dl (▽). Rats were fed a diet high in polyunsaturated fatty acids, a diet low in polyunsaturated fatty acids and Purina chow. Total specific insulin bound at each insulin concentration are means ± S.E., $n = 8$, at 800 ng/ml insulin ($P < .05$). Scatchard analysis of the effect of diet on insulin binding to the liver nuclei of control rats and diabetic rats at a blood glucose level of 400 mg/dl: Scatchard analysis was done using bound/free insulin and bound insulin. Kd was calculated using the formula Kd = −1/slope. Kd values are as follows: diabetic B/B rats fed high P/S diet, 1.6; diabetic B/B rats fed low P/S diet, 2.14; diabetic B/B rats fed chow, 2.6; control rats fed high P/S diet high affinity, 0.09 and low affinity, 0.4; control rats fed low P/S diet, 4.44; and control rats fed chow high affinity, 0.235 and low affinity, 0.483 ($P < .05$).

as the rats became diabetic. The level of 20:4(n-6) increased as the rats became diabetic (Table 2).

4. Discussion

The availability of insulin receptor for its ligand may require conformational changes in the receptor resulting from a more polyunsaturated environment [28]. These observations support earlier work in diabetic rat adipocytes on insulin binding to plasma membrane [6] and in liver nuclei from lean and obese mice [14] where feeding a high P/S diet increased insulin binding. The finding that a high-polyunsaturated-fat diet improved insulin binding suggests that a more-polyunsaturated environment may be associated with increased number of available binding sites. The molecular mechanism by which a more-polyunsaturated environment improves insulin binding is not clear.

Control rats fed chow or a high P/S diet exhibit a high- or low-affinity insulin binding site, whereas control rats fed a low P/S diet exhibit only one type of binding site. It is possible that feeding a low P/S diet causes insulin resistance, which influences the type of insulin receptor binding sites. Diabetic rats exhibit only one type of binding site for all diet treatments. As the B/B diabetic rats show a clear defect in insulin action, cells of diabetic rats bound less insulin than their respective control rats with the range of insulin concentration tested. This might be responsible for the presence of only one type of binding site in these rats.

Reports for other tissues and membranes suggest decrease in Δ9 and Δ6 desaturation in the diabetic state [29, 30]. Nuclear membrane from diabetic animals was associated with an increase in 18:2(n-6) and a decrease in 20:4(n-6) in the phosphatidylcholine fraction as the rats became more diabetic, suggesting a decrease in Δ6 desaturase activity. The opposite trend characterizes the changes occurring in phosphatidylinositol, a quantitatively minor constituent, but having extensive potential importance in signaling pathways. It has been suggested by Chanussot et al. [31] that levels of linoleic acid and oleic acid increased in B/B diabetic rats compared with control rats. It is possible that change in the phospholipid fatty acid profile of liver nuclei in B/B rats might result from an absolute or relative deficiency of insulin.

Feeding different dietary fats alters the composition of membrane constituents important in signaling. Insulin binding to liver nuclei at the onset of diabetes in B/B rats is comparable to control rats. As insulin levels decrease in highly diabetic rats, insulin binding to receptors also decreases. Insulin binding to nuclei is known to regulate gene expression for several lipogenic enzymes [13]. Insulin receptors may also translocate to the nucleus from the plasma [32]. Insulin accumulates in the nucleus of target cells [12,33]; however, the mechanism by which insulin enters the nucleus has not been determined. It has also been reported that the plasma membrane receptor was not translocated to the nucleus [34], and insulin accumulation in the nucleus continued to increase at concentrations of insulin in excess of those that saturate plasma membrane receptors [35]. These observations suggest that a non-receptor-mediated process also might be involved in nuclear translocation of insulin. These authors concluded that the increase in gene transcription observed was independent of insulin binding to plasma membrane receptors and plasma membrane-me-

Table 2
Effect of diet treatment on the fatty acid composition of phosphatidylcholine and phosphatidylinositol of liver nuclei

Fatty acid	Reference			High P/S diet			Low P/S diet			Pooled S.E.M.
	Control	Diabetic (100 mg/dl blood glucose)	Diabetic (400 mg/dl blood glucose)	Control	Diabetic (100 mg/dl blood glucose)	Diabetic (400 mg/dl blood glucose)	Control	Diabetic (100 mg/dl blood glucose)	Diabetic (400 mg/dl blood glucose)	
Phosphatidylcholine										
SFA	43.1^{b}	44.7^{b}	41.4^{b}	44.9^{b}	49.3^{a}	45.8^{b}	45.4^{b}	49.0^{a}	46.2^{ab}	1.47
MUFA	7.9^{a}	8.1^{ab}	8.3^{ab}	3.5^{d}	2.5^{d}	2.1^{d}	5.0^{c}	2.6^{d}	2.0^{d}	0.38
18:2(n-6)	14.2^{b}	16.8^{b}	23.2^{a}	12.6^{b}	10.6^{b}	13.5^{b}	11.6^{b}	12.5^{b}	14.9^{b}	1.90
20:4(n-6)	22.6^{b}	17.1^{c}	12.5^{d}	32.4^{a}	30.05^{a}	31.4^{a}	30.3^{a}	27.2^{a}	28.0^{a}	1.84
22:6(n-3)	4.5^{bc}	5.4^{b}	7.6^{a}	2.6^{c}	2.2^{c}	3.2^{bcd}	3.4^{bcd}	3.4^{bcd}	4.6^{bc}	0.66
Phosphatidylinositol										
SFA	45.1^{a}	46.6^{a}	47.0^{ab}	48.5^{ab}	47.8^{ab}	47.5^{ab}	42.0^{c}	43.7^{c}	52.9^{d}	2.23
MUFA	7.5^{a}	3.3^{cd}	2.4^{cd}	11.0^{f}	12.7^{f}	5.0^{b}	5.2^{b}	7.4^{a}	1.6^{c}	0.51
18:2(n-6)	8.3^{b}	11.6	7.5^{bc}	4.6^{cdef}	6.1^{bcd}	2.9^{ef}	5.9^{bcde}	4.1^{cdef}	2.4^{ef}	1.07
20:4(n-6)	29.4^{bc}	32.2^{abc}	35.7^{ab}	23.9^{c}	29.0^{bc}	34.3^{ab}	34.7^{ab}	38.9^{a}	37.2^{ab}	2.52
22:6(n-3)	5.5^{a}	2.7^{b}	2.7^{b}	2.9^{b}	2.0^{b}	2.3^{b}	4.7^{a}	0.8^{c}	0.7^{c}	0.55

Values are means ± S.E., $n = 8$. Only major fatty acids are reported. Values in a row (high and low P/S) without a common superscript are significantly different, $P < .05$. MUFA, monounsaturated fatty acids; P/S, polyunsaturated to saturated ratio; SFA, saturated fatty acids.

diated generation of receptor-related signaling mechanisms. Insulin has been found to induce the phosphorylation of lamin C and A isoforms and several other DNA-binding proteins in 3T3-FUU2 A-cells [36] and an isolated nuclei [13]. Recent evidence also indicates the presence of an insulin response element in the promoter of the fatty acid synthase gene in preadipocytes [37].

The present study suggests that insulin reaches the nuclei in a target tissue and binds to insulin receptors on the nuclear membrane. Diet-induced change in this binding may be associated with specific change in membrane phospholipid components. It is conceivable that insulin is internalized after binding to the nuclear membrane receptor to stimulate gene expression directly or perhaps through a kinase [13]. The molecular basis for each event that occurs after the binding of insulin to its nuclear receptor to alter insulin-responsive gene expression remains to be examined.

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