Original Article

Exposure to Marine Food Pollutants as a Risk Factor for Hypoinsulinemia and Type 2 Diabetes

Philippe Grandjean[a,b], Jan Erik Henriksen[c], Anna L. Choi[b], Maria Skaalum Petersen[d], Christine Dalgård[a], Flemming Nielsen[a], and Pal Weihe[d]

From the [a]Institute of Public Health, University of Southern Denmark, Odense, Denmark; the [b]Department of Environmental Health, Harvard School of Public Health, Boston, MA, USA; the [c]Department of Endocrinology, Odense University Hospital, Odense, Denmark; and the [d]Faroese Hospital System, Torshavn, Faroe Islands.

Correspondence: Philippe Grandjean, MD. 401 Park Drive, Room 3-110, Landmark Center, East, P.O. Box 15967, Boston, MA, 02215, USA. Tel: 617-384-8907, Fax: 617-384-8994, pgrand@hsph.harvard.edu

Acknowledgements

The study was supported by a grant from the National Institute of Health (ES013692) and the European Commission Sixth Framework Programme for RTD (FOOD-CT-2006-016253, PHIME).

Abstract

Background

Some persistent environmental chemicals are suspected of causing an increased risk of type 2 diabetes mellitus, a disease particularly common after age 70. This concern was examined in a cross-sectional study of elderly subjects from a fishing population with elevated contaminant exposures from seafood species high in the food chain.

Methods

Clinical examinations of 713 Faroese residents aged 70-74 years (64% of eligible population) included fasting plasma concentrations of glucose and insulin, and glycosylated hemoglobin. Lifetime exposure to persistent environmental chemicals from pilot whale and other traditional food was estimated from a dietary questionnaire and by analysis of blood samples for polychlorinated biphenyls (PCBs) and related food contaminants.

Results

Septuagenarians with type 2 diabetes or impaired fasting glycemia tended to have higher PCB concentrations and higher past intakes of traditional food, especially during childhood and adolescence. In non-diabetic subjects, the fasting insulin concentration decreased by 7% (95% CI: -12, -2.1) for each doubling of the PCB concentration after adjustment for sex and body mass index at age 20. Conversely, the fasting glucose concentration increased by 6%; (95% CI: -1, 13) for each doubling in PCB. Similar associations were seen in subjects without impaired fasting glycemia, while further adjustment for current body mass index and lipid metabolism parameters attenuated some of the associations.

Conclusions

Impaired insulin secretion appears to constitute an important part of the type 2 diabetes pathogenesis associated with exposure to persistent lipophilic food contaminants.

Introduction

The increasing prevalence of type 2 diabetes mellitus has been linked primarily to obesity and lack of exercise.[1] Experimental evidence suggests that exposure to dioxins and other persistent halogenated chemicals may result in both insulin resistance and disruption of insulin secretion.[2] Epidemiologic studies also support a role for environmental chemicals in the etiology of type 2 diabetes. Thus, in subjects with high-level exposure to dioxins and related substances, an increased risk of developing diabetes has been documented, in some cases more clearly in women.[3-5] Within the general population, diabetes patients have higher serum concentrations of persistent, lipophilic pollutants, including polychlorinated biphenyls (PCBs) and the pesticide metabolite 2,2-bis(4-chlorophenyl)-1,1-dichloroethene (DDE).[6-11] In addition, subjects with impaired fasting glycemia also tend to have higher cumulated pollutant concentrations in serum.[12,13] Adult Greenland Inuit showed an inverse relation between the serum PCB concentration and the insulin concentration at two hours after glucose challenge.[14] Likewise, in US veterans with existing diabetes, fasting insulin concentrations decreased at higher exposures.[15] However, non-diabetic veterans at an average age of 53 years showed higher fasting serum-insulin concentrations at increased dioxin exposure levels.[9]

The pathogenesis of type 2 diabetes may originate decades before the clinical diagnosis is made.[16] Thus, some general population studies have analyzed banked serum samples obtained before the appearance of clinical abnormalities.[9,17] Still, as the elimination half-time of dioxins, certain PCB congeners and DDE may be as long as 10 years,[18] concurrent serum concentrations may provide a reasonable estimate of cumulated past exposures. However, these lipophilic pollutants accumulate in fat tissue, and subjects with a high body mass index (BMI) therefore dilute their body burden within a larger distribution volume, while storage in body fat on the

other hand leads to a longer retention of the chemicals.[19] Still, the elimination half-life as such does not seem to be affected by the development of type 2 diabetes.[20] In addition, the known BMI-associated increase in diabetes risk has been found to be greater in subjects with an increased serum concentration of the pollutants,[8] thus calling for assessment of possible interactions.

In searching for clues to shed new light on the possible etiologic role of lipophilic pollutants, we have examined abnormalities of glucose metabolism in Faroese elderly with a high risk of diabetes development. The Faroe Islands community offers a unique research opportunity, as pollutant exposures primarily originate from traditional diets that include seabirds and blubber of the pilot whale. Past exposures may therefore be assessed by dietary questionnaire as well as by serum analysis.

Methods

Study design and subjects

A cohort of 1131 residents of the Faroe Islands aged 70-74 years was formed based on information obtained from the residents' registry. The Faroese reside on a dozen different islands in the Northern Atlantic between Norway and Iceland; about 80 % live on four northern islands connected by tunnel or bridge. Access to the examination clinic at National Hospital in Tórshavn is therefore rather easy for most subjects. Transportation costs were reimbursed. The oldest subjects were invited first for the examinations, which took place over a 12-month period. A total of 713 subjects were examined (64% of the eligible population, excluding 14 deceased).[21] Birth weights were extracted from the midwife charts kept at the Faroese National Archives.

Procedures

Up to six septuagenarians per day arrived at the clinic before breakfast and, as the first procedure, fasting blood samples were obtained. All subjects then underwent a thorough physical examination that included measurement of body weight and height.

A capillary blood sample was used to determine the plasma-glucose concentration, using the Precision Xceed instrument from MediSense and the Abbott Precision Xtra Plus strips. The blood value was automatically transformed to the fasting plasma glucose concentration by multiplying by 1.12. A fasting whole blood sample obtained by venipuncture was used to determine the relative concentration of glycosylated hemoglobin (Hb1Ac) by high-pressure liquid chromatography. Plasma was used for determination of the fasting insulin concentration by time-resolved fluoroimmunoassay (AutoDelfia, PerkinElmer Life and Analytical Sciences, Walla Oy, Turku, Finland). Serum total cholesterol and triglycerides were measured by an enzymatic reaction (Modular P, Roche Diagnostics Ltd.). The analytical imprecision of these assays was <5 percent.

Subjects not previously diagnosed with type 2 diabetes mellitus were considered incident (new) cases if the fasting plasma glucose exceeded 6.9 mmol/L and/or if the HbA1c exceeded 6.4 %. Further, subjects were classified as having impaired fasting glycemia if their fasting plasma glucose was between 6.1 and 6.9 mmol/L.[22] Insulin resistance was estimated by the homeostasis model assessment from fasting plasma insulin (FPI) and fasting plasma glucose (FPG) concentrations as FPI * FPG / 22.5, while the beta cell function was expressed as the beta index of (20 * FPI) / (FPG * 3.5).[22]

Cumulated exposures to major marine contaminants were assessed from analysis of blood samples. Serum was analyzed for persistent organochlorine pollutants by gas chromatography

using a dual capillary column system with micro-electron capture detection after solid phase extraction.[23] To avoid problems with congeners not assessed and concentrations below the detection limit, a simplified total PCB concentration was calculated as the sum of congeners CB-138, CB-153 and CB-180 multiplied by 2.[23] This analysis also provided the concentrations of DDE. Other environmental chemicals were also detected, but due to their much lower concentrations and their close correlations with PCB and DDE, these additional analytes were not examined further. As the organochlorine substances are lipophilic, PCB and DDE concentrations were expressed in relation to the total lipid concentration in the serum, as determined by a kit from Cypress Diagnostics (Langdorp, Belgium). For comparison, we also measured the blood-mercury concentration by atomic absorption as a known indicator of recent seafood intake.[24]

Current health and past medical history, including medication, were recorded by structured interview. A dietary questionnaire was used to ascertain the intake of traditional and other food during childhood and adolescence, adulthood, and the most recent year. The questionnaire focused on the amount of local food items, such as fish, whale meat and blubber, and seabirds in the diet.[25] Other risk factors of possible relevance (such as smoking and alcohol use, and body weight at age 20) were also recorded.

The study protocol was approved by the ethical review committee serving the Faroe Islands and by the Institutional Review Board at Harvard School of Public Health.

Statistical analyses

Exposure data were first treated as continuous variables, as logarithmic transformations reasonably approached a Gaussian distribution. Effects of contaminant exposures on outcome parameters were determined using standard regression techniques. Insulin concentrations and

calculated insulin resistance and beta index were also log transformed to obtain normally distributed residuals with a homogeneous variance. Sex and BMI were considered obligatory covariates in the regression analyses. BMI at age 20 was considered more relevant to lifetime contaminant exposure and diabetes. The current BMI was included as an additional covariate in separate analyses along with lipid metabolism parameters (serum triglycerides, total cholesterol, and current use of lipid reducing medication). Age, current and past smoking (yes/no), and birth weight were also considered, but none of these covariates was associated with the outcome at a $p < 0.1$ and were therefore disregarded. Exposure parameters were entered into the model, one at a time.

An odds ratio (OR) with the 95% confidence interval (CI) was calculated for the effect of a doubling of the contaminant exposure on the probability of having impaired fasting glycemia and type 2 diabetes (incident and known cases combined). We used multinomial logistic regression to assess the log odds for impaired fasting glycemia and diabetes relative to the normal status (referent group) for a unit change in the continuous predictor, or to the referent group for categorical variables.[26,27] Associations with glucose metabolism variables were assessed by multiple regression analysis. Because of the logarithmic transformation of the PCB concentration, effects were expressed as the relative change of the outcome variables per doubling of the exposure, as calculated from the antilog of the regression coefficient, with the 95% CI. Residual plots were used to assess the model fit, and the possible significance of second and third order terms was determined.

As an additional exposure variable we used the questionnaire information on traditional diet, i.e., pilot whale meat and blubber, and seabirds, during childhood and adolescence, adulthood, and the past year (fish is not an important source of PCB and DDE in this population).

As three different dietary sources were considered, standard regression analysis with confounder adjustment was complemented by structural equation models to assess the association between the overall intake of traditional food at each of the three life stages. This approach has only recently been introduced in epidemiological research, but detailed instructions are available.[28,29] We considered the observed variables to be manifestations of a single latent variable (which cannot be observed but can be estimated from the variables recorded). Each of the dietary parameters depends linearly on the latent exposure variable; error terms for the three parameters are assumed to be independent, although local dependence can be modeled. The relationship between each latent diet variable and the outcomes was then considered after adjustment for the effects of covariates. In addition to avoiding multiple comparison problems, this method can adjust for exposure imprecision and missing data, which may not be adequately addressed by standard regression analyses.[29]

Descriptive analyses and regression models were carried out in SAS (version 9.1; SAS Institute Inc., Cary, NC, USA) and R (version 2.10; http://www.R-project.org). SEMs were developed in Mplus (version 3.1l Muthen & Muthen, Los Angeles, CA, USA).

Results

One subject was excluded from the analyses due to the presence of alcoholic pancreatitis. Of the 712 remaining cohort members, a total of 168 septuagenarians (24% of the cohort) had type 2 diabetes, of whom 91 (13%) had been previously diagnosed with the disease, and 77 incident cases (11 %) were identified in the present study. Men were more likely to be diabetic (93 out of 360, or 26%) than women (75 out of 352, or 21%). Impaired fasting glycemia was found in 78 additional subjects (11%), of whom 36 were men and 42 women. The overall characteristics of

the septuagenarians are shown in Table 1. Two subjects with birth weights of 1500 and 1700 g were considered outliers and were excluded from the calculations of the effects of birth weight (all other birth weights were 2500 g and above).

All subjects had eaten traditional food to some degree, notably pilot whale meat and blubber, and seabirds, such as fulmar and puffin. The intake was particularly frequent in childhood and adolescence (from mid-1930s to early 1950s), somewhat lower in adulthood and substantially lower during the last year. Serum PCB concentrations covered a range from a background level of 0.7 μg/g lipid to a high level of 70 μg/g lipid, thus spanning a 100-fold range. DDE averaged slightly less than half of the PCB concentration, and the two were highly correlated ($r = 0.81$ after log transformation). Both were associated with traditional food intake at different ages, while blood-mercury showed close correlation with recent whale meat intake (Table 2).

Of the risk factors considered, BMI was by far the most important predictor of type 2 diabetes and impaired glucose metabolism (Table 3). PCB correlated poorly with the current BMI ($r = -0.003$) and much better with the BMI at age 20 ($r = 0.28$). Mercury concentrations were associated with PCB and DDE, but not with diabetes or the risk factors.

After adjustment for sex and BMI at age 20, contaminant exposures and childhood/adolescence diet were associated with an increased risk of IFG and type 2 diabetes, although addition of further covariates somewhat weakened some of the associations (Table 4). While all of the odds ratios were above 1, most estimates had fairly wide confidence intervals. The diet during adulthood showed less stable associations. No difference was apparent in these associations between men and women, and inclusion of an interaction parameter between BMI and PCB barely changed the results (data not shown).

Fasting plasma concentrations of insulin decreased at higher PCB levels in all non-diabetic subjects as well as in subjects with normal glucose tolerance (Table 5, Fig. 1). The calculated beta index showed a similar association, but in diabetic subjects, fasting insulin concentrations did not show any clear trend (data not shown). Glucose concentrations increased at higher exposures. If further adjusted for the calculated insulin resistance, this positive association was strengthened (data not shown). On the other hand, glycosylated hemoglobin did not reveal any obvious effect of the exposure. Associations with DDE and childhood/adolescence diet were in accordance with the PCB results, although effect estimates were smaller and less stable (data not shown).

Discussion

This cross-sectional study of a population-based sample of Faroese septuagenarians showed that type 2 diabetes and impaired fasting glycemia were associated with increased serum PCB concentrations and with a history of more frequent intake of traditional food in childhood and adolescence. In healthy subjects, increased PCB exposure was associated with a decreased fasting insulin concentration and increased fasting glucose. These findings support a possible effect of exposure to organochlorine substances on the pathogenesis of type 2 diabetes.

A major strength of this population-based study is the high participation rate among the elderly subjects within a narrow age range. The total prevalence of diabetes in the population sample was high (24%). US data suggest a prevalence of about 16% for subjects above 65 years, with fasting glucose suggesting an additional 6% having undiagnosed type 2 diabetes,[30] although somewhat less if based on glycosylated hemoglobin.[31] Among the Faroese septuagenarians, slightly more than half had been previously diagnosed with type 2 diabetes, and the clinical

examination therefore discovered a considerable number of new cases. In addition, 11% had impaired fasting glycemia.

The serum PCB concentrations are highly elevated in this population. The main source is pilot whale blubber; with an average PCB concentration of about 20 μg/g,[32] an estimated average intake of 50 g per week would result in a weekly PCB intake of 1 mg. A PCB exposure of similar magnitude was calculated for highly-exposed Great Lakes anglers at an average annual level of 46.5 mg.[33] Because of the long elimination half-life of major PCB congeners, the serum PCB concentration represents a long-term accumulation, although the serum concentration and the total body burden depend on the size of the lipid compartment. Further, the capability of PCB elimination is greater in slim subjects.[18] Thus, the impacts of BMI on serum PCB concentrations and the greater diabetes risk in obese subjects may cloud any diabetogenic effect reflected by the current serum PCB concentration.

Stronger evidence of an increased diabetes risk appears from the decreasing fasting insulin concentrations at higher PCB concentrations in subjects without diabetes or without impaired fasting glycemia. The concomitant increase in fasting glucose supported this tendency. These observations are in accordance with possible beta cell toxicity or exhaustion, a mechanism supported by experimental toxicity studies, although insulin releases increases in the short term.[2] Decreased fasting plasma-insulin in highly exposed subjects has been reported previously,[14,15] although a positive association with increased dioxin exposures has been observed in younger adults.[9,12]

The apparently discrepant findings should be considered in the light of different stages of the pathogenesis of type 2 diabetes mellitus, thought to initially include a phase of increasing insulin resistance, which is compensated for by an increase in insulin secretion to maintain

normal plasma glucose concentrations. With time, exhaustion of the beta cells develops as a result of the insulin resistance, perhaps coupled with impacts of toxic influences.[34] Thus, our results suggest that organochlorine exposure may be associated with both insulin insensitivity and beta cell toxicity, in agreement with experimental studies.[2] Several mechanisms of beta cell toxicity may be involved, including activation by dioxin-related substances of the intranuclear aryl hydrocarbon receptor.[35]

Previous observations of dioxin-associated increases in fasting plasma insulin concentrations in younger subjects would fit with a pattern of initial stimulation of insulin secretion, perhaps triggered by early insulin insensitivity. The increased fasting insulin concentration in exposed adults may later, as observed in the septuagenarians examined in the present study, be reversed as a sign of beta cell depletion. This change could be a further step in the development of type 2 diabetes mellitus, especially if coinciding with obesity. Because all of the underlying epidemiological data originate from cross-sectional studies, and not from populations prospectively followed through decades, further research is needed to elucidate the role of organochlorine pollutants in human diabetes pathogenesis.

The possible role of individual or groups of PCB congeners in diabetes etiology cannot be ascertained from this study due to the mixed exposure and the high correlation between their serum concentrations. Although some PCB congeners have dioxin-like toxicity properties, the overall dioxin exposure in the Faroe Islands appears not to be increased above the northern European average.[36] The DDE concentrations are also elevated, but exposures to chlorinated pesticides other than DDE are comparatively low. In a US population-based study, serum concentrations of six different pollutants detectable in at least 80% of the participants showed an adjusted odds ratio of 38 for diabetes in subjects in the highest exposure quintile, as compared

with those in the lowest.[8] All pollutants seemed to contribute to the increased risk, but all of them are also highly persistent, so that they serve as markers of past and cumulated exposures to environmental chemicals, some of which may have been eliminated over time. Among possible candidates as diabetogenic substances are the brominated flame retardants,[37] but exposures to many of these persistent pollutants are interrelated. Increased consumption of oily fish, which may contain lipophilic contaminants, seems not to reduce the risk of diabetes, and high intakes may increase the incidence of this disease,[38] thus perhaps again suggesting a possible role of lipophilic seafood contaminants. At this point, the identity and specific effects of the potentially causative substance(s) remains unclear. The relatively small odds ratios observed in the highly-exposed Faroese subjects indicate that a possible diabetes risk may not increase linearly with the dose. However, the odds ratios may have been affected by the small number of subjects with low exposure.

An important weakness is that the single measurement of PCBs at age 70-74 years is likely to be an imprecise indicator of the life-time exposure to the causative contaminants, in part because changes in BMI may impact on the serum PCB concentrations. Such imprecision would tend to cause a bias towards the null and underestimate the effect. The same applies to the use of questionnaire answers on past diets, which is exacerbated by the absence of information on past concentrations of contaminants in traditional food. The discovery of PCBs and other persistent organic pollutants in the environment dates back to the 1970s, but sediment analyses suggest that substantial amounts were already passing into global food chains during the 1950s.[39,40] The septuagenarians examined in this study were therefore exposed to these substances since childhood.

The increasing world-wide prevalence of type 2 diabetes is in part a result of an increased survival of the diabetic population due to improved treatment, but it is also a result of unhealthy, sedentary lifestyle and an energy-dense diet, both resulting in an increased body weight.[1] If environmental pollutants play a role in triggering type 2 diabetes, prevention may need to involve mechanisms other than individual intervention, as these substances are generally invisible to the consumer and may be difficult to address within current health care systems. The contaminants studied so far have now largely been banned, and exposures to these contaminants have recently decreased. However, subjects now approaching the age with the greatest diabetes incidence have been born at a time when environmental accumulation of these pollutants was at a peak, and the developmental programming of glucose metabolism may therefore have been affected by diabetogenic chemicals.[16] Intensified prevention of exposure to environmental pollutants should be considered as an attractive means of complementing preventive efforts against type 2 diabetes.

References

1. World Health Organization (WHO). Diabetes programme. Vol. 2010 World Health Organization (WHO), 2010.
2. Ruzzin J, Petersen R, Meugnier E, Madsen L, Lock EJ, Lillefosse H, Ma T, Pesenti S, Sonne SB, Marstrand TT, Malde MK, Du ZY, Chavey C, Fajas L, Lundebye AK, Brand CL, Vidal H, Kristiansen K, Froyland L. Persistent organic pollutant exposure leads to insulin resistance syndrome. *Environ Health Perspect.* 2010;118:465-471.
3. Consonni D, Pesatori AC, Zocchetti C, Sindaco R, D'Oro LC, Rubagotti M, Bertazzi PA. Mortality in a population exposed to dioxin after the Seveso, Italy, accident in 1976: 25 years of follow-up. *Am J Epidemiol.* 2008;167:847-858.
4. Henriksen GL, Ketchum NS, Michalek JE, Swaby JA. Serum dioxin and diabetes mellitus in veterans of Operation Ranch Hand. *Epidemiology.* 1997;8:252-258.
5. Wang SL, Tsai PC, Yang CY, Leon Guo Y. Increased risk of diabetes and polychlorinated biphenyls and dioxins: a 24-year follow-up study of the Yucheng cohort. *Diabetes Care.* 2008;31:1574-1579.
6. Fierens S, Mairesse H, Heilier JF, De Burbure C, Focant JF, Eppe G, De Pauw E, Bernard A. Dioxin/polychlorinated biphenyl body burden, diabetes and endometriosis: findings in a population-based study in Belgium. *Biomarkers.* 2003;8:529-534.
7. Lee DH, Lee IK, Porta M, Steffes M, Jacobs DR, Jr. Relationship between serum concentrations of persistent organic pollutants and the prevalence of metabolic syndrome among non-diabetic adults: results from the National Health and Nutrition Examination Survey 1999-2002. *Diabetologia.* 2007;50:1841-1851.
8. Lee DH, Lee IK, Song K, Steffes M, Toscano W, Baker BA, Jacobs DR, Jr. A strong dose-response relation between serum concentrations of persistent organic pollutants and diabetes: results from the National Health and Examination Survey 1999-2002. *Diabetes Care.* 2006;29:1638-1644.
9. Longnecker MP, Michalek JE. Serum dioxin level in relation to diabetes mellitus among Air Force veterans with background levels of exposure. *Epidemiology.* 2000;11:44-48.
10. Rignell-Hydbom A, Rylander L, Hagmar L. Exposure to persistent organochlorine pollutants and type 2 diabetes mellitus. *Hum Exp Toxicol.* 2007;26:447-452.
11. Turyk M, Anderson H, Knobeloch L, Imm P, Persky V. Organochlorine exposure and incidence of diabetes in a cohort of Great Lakes sport fish consumers. *Environ Health Perspect.* 2009;117:1076-1082.
12. Cranmer M, Louie S, Kennedy RH, Kern PA, Fonseca VA. Exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is associated with hyperinsulinemia and insulin resistance. *Toxicol Sci.* 2000;56:431-436.
13. Kern PA, Said S, Jackson WG, Jr., Michalek JE. Insulin sensitivity following agent orange exposure in Vietnam veterans with high blood levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin. *J Clin Endocrinol Metab.* 2004;89:4665-4672.
14. Jørgensen ME, Borch-Johnsen K, Bjerregaard P. A cross-sectional study of the association between persistent organic pollutants and glucose intolerance among Greenland Inuit *Diabetologia.* 2008;51:1416-1422.
15. Michalek JE, Akhtar FZ, Kiel JL. Serum dioxin, insulin, fasting glucose, and sex hormone-binding globulin in veterans of Operation Ranch Hand. *J Clin Endocrinol Metab.* 1999;84:1540-1543.

16. Jones RH, Ozanne SE. Fetal programming of glucose-insulin metabolism. *Mol Cell Endocrinol.* 2009;297:4-9.
17. Rignell-Hydbom A, Lidfeldt J, Kiviranta H, Rantakokko P, Samsioe G, Agardh CD, Rylander L. Exposure to p,p'-DDE: a risk factor for type 2 diabetes. *PLoS One.* 2009;4:e7503.
18. Grandjean P, Budtz-Jorgensen E, Barr DB, Needham LL, Weihe P, Heinzow B. Elimination half-lives of polychlorinated biphenyl congeners in children. *Environ Sci Technol.* 2008;42:6991-6996.
19. Wolff MS, Anderson HA. Polybrominated biphenyls: Sources and disposition of exposures among Michigan farm residents, 1976-1980. *Eur. J. Oncol.* 1999;4:645-651.
20. Michalek JE, Ketchum NS, Tripathi RC. Diabetes mellitus and 2,3,7,8-tetrachlorodibenzo-p-dioxin elimination in veterans of Operation Ranch Hand. *J Toxicol Environ Health A.* 2003;66:211-221.
21. Dalgard C, Petersen MS, Schmedes AV, Brandslund I, Weihe P, Grandjean P. High latitude and marine diet: vitamin D status in elderly Faroese. *Br J Nutr.* 2010;104:914-918.
22. Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. *Diabetes Care.* 2004;27:1487-1495.
23. Heilmann C, Grandjean P, Weihe P, Nielsen F, Budtz-Jorgensen E. Reduced antibody responses to vaccinations in children exposed to polychlorinated biphenyls. *PLoS Med.* 2006;3:e311.
24. Grandjean P, White RF, Weihe P, Jorgensen PJ. Neurotoxic risk caused by stable and variable exposure to methylmercury from seafood. *Ambul Pediatr.* 2003;3:18-23.
25. Petersen MS, Halling J, Bech S, Wermuth L, Weihe P, Nielsen F, Jorgensen PJ, Budtz-Jorgensen E, Grandjean P. Impact of dietary exposure to food contaminants on the risk of Parkinson's disease. *Neurotoxicology.* 2008;29:584-590.
26. Agresti A. Categorical data analysis. Wiley series in probability and statistics. 2nd ed. New York, NY: John Wiley & Sons, 2002.
27. Hosmer DW, Lemeshow S. Applied logistic regression. Wiley series in probability and statistics. Texts and references section. 2nd ed. New York: Wiley, 2000.
28. Bollen KA. Structural equations with latent variables. New York: John Wiley, 1989.
29. Budtz-Jorgensen E, Keiding N, Grandjean P, Weihe P. Estimation of health effects of prenatal methylmercury exposure using structural equation models. *Environ Health.* 2002;1:2.
30. Cowie CC, Rust KF, Byrd-Holt DD, Eberhardt MS, Flegal KM, Engelgau MM, Saydah SH, Williams DE, Geiss LS, Gregg EW. Prevalence of diabetes and impaired fasting glucose in adults in the U.S. population: National Health And Nutrition Examination Survey 1999-2002. *Diabetes Care.* 2006;29:1263-1268.
31. Cowie CC, Rust KF, Byrd-Holt DD, Gregg EW, Ford ES, Geiss LS, Bainbridge KE, Fradkin JE. Prevalence of diabetes and high risk for diabetes using A1C criteria in the U.S. population in 1988-2006. *Diabetes Care.* 2010;33:562-568.
32. Borrell A, Aguilar A. Pollution by DDT and PCB in blubber and muscle of long-finned pilot whales from the Faroe Islands. In: Donovan GP, Lockyer CH, Martin AR, eds. Biology of northern hemisphere pilot whales Vol. Special Issue 14. Cambridge: International Whaling Commission, 1993; 351-367.

33. Anderson HA. General population exposure to environmental concentrations of halogenated biphenyls. In: Kimbrough RD, Jensen AA, eds. Halogenated biphenyls, terphenyls, naphthalenes, dibenzodioxins and related products. 2nd ed. Amsterdam: Elsevier 1989; 325-380.
34. Beck-Nielsen H, Vaag A, Poulsen P, Gaster M. Metabolic and genetic influence on glucose metabolism in type 2 diabetic subjects--experiences from relatives and twin studies. *Best Pract Res Clin Endocrinol Metab.* 2003;17:445-467.
35. Kurita H, Yoshioka W, Nishimura N, Kubota N, Kadowaki T, Tohyama C. Aryl hydrocarbon receptor-mediated effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on glucose-stimulated insulin secretion in mice. *Journal of Applied Toxicology.* 2009;29:689-694.
36. Grandjean P, Weihe P, Needham LL, Burse VW, Patterson DG, Jr., Sampson EJ, Jorgensen PJ, Vahter M. Relation of a seafood diet to mercury, selenium, arsenic, and polychlorinated biphenyl and other organochlorine concentrations in human milk. *Environ Res.* 1995;71:29-38.
37. Lim JS, Lee DH, Jacobs DR, Jr. Association of brominated flame retardants with diabetes and metabolic syndrome in the U.S. population, 2003-2004. *Diabetes Care.* 2008;31:1802-1807.
38. Kaushik M, Mozaffarian D, Spiegelman D, Manson JE, Willett WC, Hu FB. Long-chain omega-3 fatty acids, fish intake, and the risk of type 2 diabetes mellitus. *Am J Clin Nutr.* 2009;90:613-620.
39. Eisenreich SJ, Capel PD, Robbins JA, Bourbonniere R. Accumulation and diagenesis of chlorinated hydrocarbons in lacustrine sediments. *Environ Sci Technol.* 1989;23:1116-1126.
40. Vartiainen T, Mannio J, Korhonen M, Kinnunen K, Strandman T. Levels of PCDD, PCDF and PCB in dated lake sediments in Subarctic Finland. *Chemosphere* 1997;34:1341-1350.

Figure legend

Figure 1. Generalized additive model for the serum total PCB concentration (μg/g lipid) as predictor for the average fasting plasma insulin concentration in septuagenarians without T2D and IFG, with 95% confidence limits, after adjustment for sex and BMI at age 20 years.

Table

Table 1. Characteristics of 712 Faroese subjects aged 70-74 years. Means (with standard deviation in parenthesis) are listed for normally distributed parameters, otherwise the geometric mean (with 50% range in parenthesis).

Variable	N	All	Men (N=360)	Women (N=352)
Age at examination (years)	712	72.4 (1.2)	72.4 (1.2)	72.5 (1.1)
Sex (men / women) [%]	712	50.7 / 49.3	-	-
Current smoking (no / yes) [%]	709	82 / 18	84/16	81/19
Past smoking (no / yes) [%]	709	33 / 67	20/80	46/54
Body weight (kg)	710	80.8 (15.0)	87.5 (13.6)	73.9 (13.3)
Height (cm)	709	167 (8.5)	173 (5.5)	161 (5.7)
Body mass index (kg/m^2)				
Current	709	28.9 (4.7)	29.1(4.3)	28.7 (5.0)
Age 20	688	23.4 (3.2)	24.4 (3.2)	22.4 (2.9)
Number of blubber dinners per month:				
In childhood (0-1/2-4 /more than 4) [%]	706	13 / 8 / 79	10 / 8 / 82	16 / 9 / 75
In adulthood (0-1/2-4/more than 4) [%]	707	32 / 23 / 45	24 / 22 / 54	41 / 24 / 35
During recent year (0-1 / 2-4 / more than 4) [%]	709	58 / 22 /20	48 / 25 / 27	68 / 19 / 13
Serum ΣPCB (µg/g lipid)	710	8.1 (4.7-14)	11.2(7.7-18)	5.8(3.7-9.3)
Serum DDE (µg/g lipid)	710	2.9 (1.7-5.5)	3.7 (2.3-6.6)	2.3 (1.3-4.2)
Blood mercury (µg/L)	707	14 (7.2-25)	18.6 (9.8-35)	9.95 (5.5-18)
Fasting plasma-glucose (µmol/L)	695	5.7 (1.6)	5.7 (1.7)	5.7 (1.6)
Plasma fasting insulin (pmol/L)	703	40 (26-58)	41 (26-61)	39 (26-55)
Insulin resistance [a]	687	9.8 (6.1-15.5)	9.9 (6.2-16)	9.5 (6.0-14)
Beta index [a]	687	41 (27-59)	42 (27-62)	41 (27-58)
Blood glycosylated hemoglobin (%)	708	6.1 (0.7)	6.1 (0.7)	6.1 (0.7)
Serum lipids [mmol/L]				
Total cholesterol	705	5.8 (1.3)	5.5 (1.2)	6.2 (1.3)
Triglycerides	706	1.5 (0.7)	1.5 (0.7)	1.6 (0.8)

[a]Homeostasis model assessment

Table

Table 2: Correlations[a] between frequency of bird, whale, blubber, and fish meals and contaminant exposure indicators in 712 Faroese septuagenarians.

Types of food and life stage	Serum ΣPCB	Serum DDE	Blood Hg
Marine bird dinners/ month			
Childhood	0.28	0.20	0.11
Adult	0.34	0.24	0.21
last year	0.24	0.20	0.27
Blubber dinners/ month			
Childhood	0.21	0.16	0.09
Adult	0.35	0.27	0.25
last year	0.37	0.31	0.45
Whale meat dinners/ month			
Childhood	0.12	0.07	0.06
Adult	0.27	0.21	0.22
last year	0.30	0.25	0.50
Fish dinners/ week			
childhood	-0.05	-0.03	-0.04
adult	0.04	0.04	0.02
last year	0.04	0.05	0.08

[a]Spearman correlation coefficients

Table

Table 3. Unadjusted arithmetic and geometric averages of risk factors associated with type 2 diabetes mellitus (T2D) status among 712 Faroese subjects aged 70-74 years

Variable	T2D status				p-value
	Normal	Impaired fasting glycemia	Incident cases	Known cases	
Number of subjects	466	78	77	91	-
Age at examination (years)	72.4	72.6	72.2	72.6	0.86
Sex (men / women) [%]	50 / 50	47 / 53	53 / 47	58 / 42	0.41
Current smoking (no / yes) [%]	82 / 18	77 / 23	78 / 22	92 / 8	0.16
Past smoking (no / yes) [%]	36 / 64	32 / 68	23 / 77	28 / 72	0.37
Body weight (kg)	79	81	84	87	
Height (cm)	167	167	166	168	0.83
Body mass index (kg/m^2)	28.3	28.9	30.2	30.5	<0.0001
Current	23.3	22.8	23.3	24.4	0.42
Age 20					
Number of blubber dinners per month:					
In childhood (0-1 / 2-4 / more than 4) [%]	14/9/77	10/8/82	8/9/83	12/6/82	0.58
In adulthood (0-1 / 2-4 / more than 4) [%]	32/24/44	33/21/46	38/15/47	30/23/47	0.72
During recent year (0-1 / 2-4 / more than 4) [%]	55/24/21	64/18/18	66/17/17	61/22/17	0.44
Serum ΣPCB (µg/g lipid) [a]	7.8	8.2	8.8	8.9	0.09
Serum DDE (µg/g lipid) [a]	2.8	3.1	3.3	3.2	0.12
Blood mercury (µg/L)[a]	13.7	13.9	15.3	12.2	0.98
Fasting plasma-glucose (µmol/L)	5.09	6.34	6.19	7.67	<0.0001
Plasma fasting insulin (pmol/L) [a]	35.6	39.8	48.4	59.4	<0.0001
Insulin resistance [a,b]	8.00	11.1	12.9	18.6	<0.0001
Beta index [a,b]	40.6	35.6	46.6	46.5	0.28
Blood glycosylated hemoglobin (%)	5.84	5.90	6.55	7.67	<0.0001
Serum lipids [mmol/L]	6.0	6.0	5.8	5.0	<0.0001
Total cholesterol	1.4	1.6	1.7	1.8	<0.0001
Triglycerides					

[a]Geometric mean
[b]Homeostasis model assessment

Table

Table 4: Odds ratios (95% CI) of impaired fasting glycemia and type 2 diabetes mellitus[a] in 712 Faroese septuagenarians in regard to a doubling of the lipid-based serum concentrations of PCB and DDE, and frequency of traditional diet during childhood.

	PCB	DDE	Childhood traditional diet
Model 1[b]			
Impaired fasting glycemia	1.14 (0.90, 1.45)	1.11 (0.91, 1.34)	1.24 (0.81, 1.89)
Type 2 diabetes	1.10 (0.91, 1.31)	1.06 (0.92, 1.21)	1.25 (0.85, 1.84)
Model 2[c]			
Impaired fasting glycemia	1.25 (0.97, 1.61)	1.13 (0.92, 1.37)	1.07 (0.67, 1.71)
Type 2 diabetes	1.11 (0.91, 1.35)	1.01 (0.87, 1.16)	1.14 (0.75, 1.74)

[a] Both new and previously diagnosed type 2 diabetes cases; the referent outcome category being normal glucose tolerance

[b] Adjusted for sex, and BMI at 20 years of age

[c] Adjusted for sex, BMI at 20 years of age, BMI, serum triglycerides, serum total cholesterol, and lipid reducing medication

Table

Table 5: Percent change (95% confidence interval) in glucose tolerance parameters associated with a doubling in the serum ΣPCB concentration in 70-74 years old Faroese subjects with normal glucose metabolism (N = 466) or without type 2 diabetes diagnosis (N= 543).

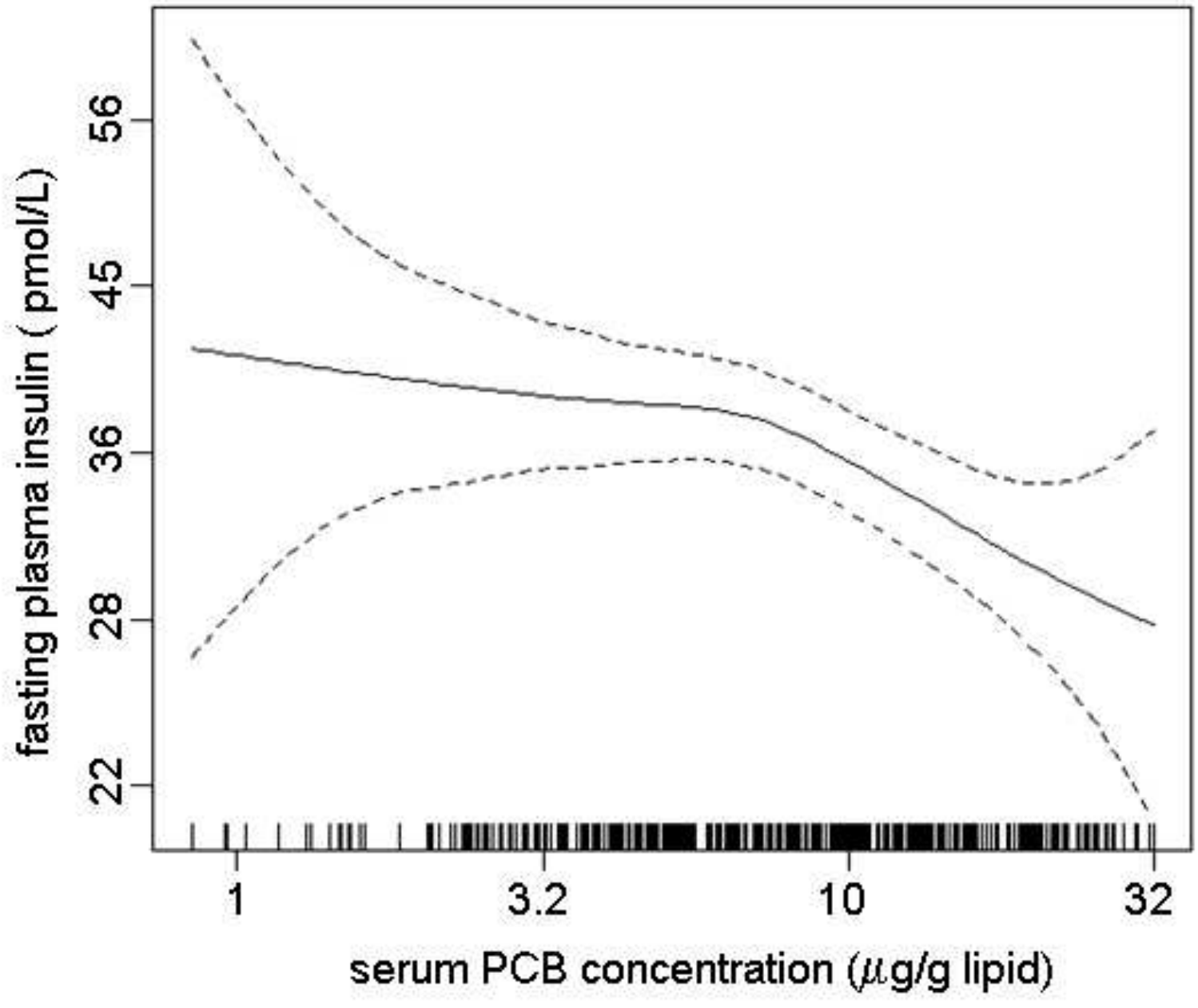
	Fasting plasma-insulin[a]	Fasting plasma-glucose[a]	Blood glycosylated hemoglobin[a]
Model 1[b]			
Non-diabetic	-7.0 (-12, -2.1)	5.8 (-0.9, 13)	-0.43 (-3.0, 2.1)
Normal	-7.1 (-12, -1.9)	3.9 (-2.5, 10)	-0.36 (-3.2, 2.4)
Model 2[c]			
Non-diabetic	-3.8 (-7.8, 0.4)	6.8 (0.01, 13.7)	-0.04 (-2.6, 2.5)
Normal	-3.2 (-7.4, 1.3)	3.8 (-2.6, 10.3)	-0.08 (-2.9, 2.7)

[a]Percent change in a multiplicative scale obtained by exponentiating the regression coefficient in the log-transformed model

[b] Adjusted for sex, and BMI at 20 years of age

[c] Also adjusted for sex, weight at BMI at 20 years, current BMI, serum triglycerides, total cholesterol, and lipid reducing medication

Figure


fasting plasma insulin (pmol/L)
22
28
36
45
56
1
3.2
10
32
serum PCB concentration (μg/g lipid)