HABILITATIONSSCHRIFT

Modifiable risk factors of cardiovascular disease from an epidemiological and a clinical perspective

Zur Erlangung der Venia Legendi der Universität Zürich

David Fäh

1. August 2009

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Worldwide, cardiovascular diseases (CVD) are the leading cause of death. Several risk factors for CVD have been established, among which obesity, diabetes, hypertension, unfavourable lipid profile, physical inactivity and smoking are the most common. Most of these risk factors depend on diet, either by an excess of caloric intake and subsequent obesity or by a direct impact of nutrients. Recently, the role of sugar has received more attention. Its consumption has strongly increased in the past decades in the US but also in many European countries, including Switzerland. This increase was particularly strong for fructose and was mainly driven by mounting consumption of sugar sweetened drinks. Much more than glucose, fructose has an immediate impact on CVD risk factors. The consumption of large amounts of fructose can increase serum concentrations of cholesterol, glucose and uric acid, and may lead to higher blood pressure. However, the most pronounced change is an increase in serum triglycerides which may be accompanied by impaired insulin sensitivity.

While CVD rates generally go down in the western world, there has recently been a sharp increase in developing countries. These regions have skyrocketing population growth and thus deserve increasing attention. Representative for the future development of many developing countries is the recent history of the Republic of the Seychelles, located in the Indian Ocean. Since the construction of the airport in the 1970s, this country has experienced an accelerated epidemiological transition from a self-sustaining population living from agriculture and fishing to a service-orientated and consuming society. Along with an increasing buying power and the adoption of a western lifestyle, tobacco and heavy alcohol use have become more and more popular. Per capita caloric intake has increased substantially in the Seychelles and the consumption of sugar sweetened drinks has tripled in the past 25 years. In Switzerland, there has also been a rise in sugar intake, but the increase was less pronounced: per capita consumption increased from 41 to 48 kilo in the past 25 years. In 2001, each Swiss inhabitant consumed in average 105 litres of sugar sweetened drinks.

Surveys are an important tool to monitor diseases and risk factors in a population. Descriptive epidemiology can also be used to generate hypothesis about causal relationships which can thereafter be tested with clinical trials. Both approaches are necessary in order to detect and explore risk factors in a population. Based on these findings, public health measures aimed at reducing population risks can be developed and implemented.

The selected six publications highlight CVD risk factors from an epidemiological and clinical perspective. Three papers explore, on a population level, the development and the interaction of CVD risk factors and behaviours among each other and with disease parameters. The other three publications elucidate the direct impact of risk and protective factors on healthy humans. The first epidemiological study shows that the strong and fast increase in obesity prevalence in the Seychelles was not only paralleled by a rise of diabetes cases but also of persons with less severe impairments of glucose metabolism, such as impaired fasting glucose and impaired glucose tolerance [1]. A large

proportion of diabetes patients were not aware of the disease and about half of all diabetes cases was attributable to overweight and obesity. In-depth analysis showed that increasing BMI and deterioration of glucose metabolism were gradually associated with increasing insulin resistance. A second study revealed that not only diabetes but also less severe impairments of glucose metabolism go along with a deterioration of common CVD risk factors including hypertension, dyslipidemia and smoking [2]. Intima-media thickness of the carotid and femoral artery can be regarded as CVD outcome since it measures irreversible organic vascular changes, described as atherosclerosis. In this publication we also found that intima-media thickness gradually increased with increasing impairment of glucose metabolism. Interestingly, a part of these associations were still significant after adjustment for common CVD risk factors. This suggests that additional factors mediate the effect of glucose metabolism impairment on intima-media thickness. The analysis also revealed that adjustment for simple measurements (BMI and waist circumference) had similar impact as adjustment for common clinical CVD risk factors. This has practical relevance in that these simple measurements are valid tools for risk assessment in a population. A novel side-finding of this analysis was that the relationships between impaired glucose metabolism and intima-media thickness tended to be larger at femoral than carotid levels independently of adjustment. Accordingly, one can speculate whether measurement at femoral site could be more useful for risk assessment.

The two studies show that CVD risk factors can rapidly increase in developing countries and may reach or exceeded levels typically found in middle- or high-income countries. This increase is mainly driven by fundamental changes in lifestyle. Simple and inexpensive risk monitoring – for example the assessment of BMI, family history of CVD and diabetes and capillary glucose level - can be as effective as much more extensive examinations. In view of the strong association, early prevention of factors leading to obesity is a cornerstone strategy to curb CVD and their risk factors.

Disadvantageous risk behaviours usually do not occur isolated. They mostly coincide with others and build clusters. This is also the case for smoking, cannabis use and heavy alcohol consumption. Youth who start with one of these behaviours frequently continue with another. This finding of the third epidemiological publication has several public health implications [3]. It is important to adopt healthy behaviours early in life since this has the potential to reduce CVD in adulthood. Furthermore, clustering of risk factors emphasizes the need to address these behaviours within a comprehensive and integrated approach.

As suggested by the epidemiological studies, sugar sweetened drinks may play an important role as CVD risk factor. This was substantiated by the first clinical study that found a strong increase in serum triglycerides following high fructose intake during a short period [4]. This increase was driven by a boosted hepatic de novo lipogenesis and accompanied by insulin resistance a potential precursor of diabetes. As a possible CVD protective factor, fish oil was thought to prevent metabolic alterations induced by fructose. This was however only the case for a part of the increase in serum triglycerides. Because the high amounts of administrated fructose and the short duration of feeding

represented a somewhat unrealistic condition, a second protocol has been developed. Fructose administration was prolonged to four weeks but the amount was halved and now corresponded to the realistic daily consumption of about 1.5 litres of soda [5]. In view of the strong increase of de novo lipogenesis, hepatic fat accumulation could be expected. Therefore, magnetic resonance spectroscopy was used in the second study to measure changes in intrahepatic fat. In healthy young men, the lower fructose dose led to increased triglyceride and glucose levels but neither to insulin resistance nor to intrahepatic fat accumulation. However, alterations in the later two were found in one healthy subject who had a first degree relative with type 2 diabetes. This subject had to be excluded from the study but constituted a basis for a consecutive study which is not discussed here.

In the second clinical study, muscle and adipose tissue biopsies were taken from each subject. With these samples, changes in expression of 16 genes involved in lipid, sugar and energy metabolism were measured. The results were published in a third paper [6]. The expression of a lipogenic enzyme was increased. This enzyme favours lipid deposition in muscles and is associated with liver steatosis and insulin resistance. In contrast, the expression of an insulin-stimulated receptor was decreased after fructose intake, which could be interpreted as first step towards insulin resistance. An activator protein that stimulates mitochondrial biogenesis also tended to be less expressed after fructose consumption. These findings suggest that an increase in fat deposition in hepatic and muscle cells could indeed be expected from fructose consumption. However, the duration of administration was probably too short for these young healthy and normal weight males without family history of diabetes.

In conclusion, the fast increase in CVD in many parts of the world is mainly due to modifiable risk factors. Particularly for rapidly developing countries, the emerging CVD epidemic is a major burden because these countries do not have sufficient resources to effectively monitor, prevent and treat CVD. Epidemiological research suggests that the consumption of sugar sweetened drinks could be a factor contributing to this increase in CVD. These drinks contain large amounts of fructose, a type of sugar whose consumption has over proportionally increased in the past years. Clinical research has shown that even amounts typically consumed can lead to metabolic deteriorations which increase CVD risk. However, the harmfulness of the effect of fructose strongly depends on the amount ingested. Dose dependence applies for most risk factors as well as the fact, that they rarely occur alone and that their impact varies significantly between individuals. Fructose is a risk factor that could particularly easily be modified because the main source are sugar sweetened drinks. A ban of distributors in schools, a stricter regulation of advertisement and a tax on sugar sweetened drinks could effectively and inexpensively reduce fructose intake. To assess changes in risk factors in a population, few simple measurements are almost as effective as extensive assessments. Inexpensive monitoring and prevention of risk factors are of major importance in resource-constrained settings as found in developing countries. From a public health perspective both epidemiological and clinical approaches are necessary to elucidate the impact of CVD risk factors and the potential for prevention.

Publications selected for habilitation

1. Faeh D, William J, Tappy L, Ravussin E, Bovet P. Prevalence, awareness and control of diabetes in the Seychelles and relationship with excess body weight. BMC Public Health. 2007;7:163.

2. Faeh D, William J, Yerly P, Paccaud F, Bovet P. Diabetes and pre-diabetes are associated with cardiovascular risk factors and carotid/femoral intima-media thickness independently of markers of insulin resistance and adiposity. Cardiovasc Diabetol. 2007;6:32.

3. Faeh D, Viswanathan B, Chiolero A, Warren W, Bovet P. Clustering of smoking, alcohol drinking and cannabis use in adolescents in a rapidly developing country. BMC Public Health. 2006;6:169.

4. Faeh D, Minehira K, Schwarz JM, Periasamy R, Park S, Tappy L. Effect of fructose overfeeding and fish oil administration on hepatic de novo lipogenesis and insulin sensitivity in healthy men. Diabetes. 2005;54(7):1907-13.

5. Le KA, Faeh D, Stettler R, Ith M, Kreis R, Vermathen P, et al. A 4-wk high-fructose diet alters lipid metabolism without affecting insulin sensitivity or ectopic lipids in healthy humans. Am J Clin Nutr. 2006;84(6):1374-9.

6. Le KA, Faeh D, Stettler R, Debard C, Loizon E, Vidal H, et al. Effects of four-week high-fructose diet on gene expression in skeletal muscle of healthy men. Diabetes Metab. 2008;34(1):82-5.

BMC Public Health

Research article

Prevalence, awareness and control of diabetes in the Seychelles and relationship with excess body weight

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Abstract

Background: The evidence for a "diabesity" epidemic is accumulating worldwide but population-based data are still scarce in the African region. We assessed the prevalence, awareness and control of diabetes (DM) in the Seychelles, a rapidly developing country in the African region. We also examined the relationship between body mass index, fasting serum insulin and DM.

Methods: Examination survey in a sample representative of the entire population aged 25–64 of the Seychelles, attended by 1255 persons (participation rate of 80.2%). An oral glucose tolerance test (OGTT) was performed in individuals with fasting blood glucose between 5.6 and 6.9 mmol/l. Diabetes mellitus (DM), impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) were defined along criteria of the ADA. Prevalence estimates were standardized for age.

Results: The prevalence of DM was 11.5% and 54% of persons with DM were aware of having DM. Less than a quarter of all diabetic persons under treatment were well controlled for glycemia (HbA1c), blood pressure or LDL-cholesterol. The prevalence of IGT and IFG were respectively 10.4% and 24.2%. The prevalence of excess weight (BMI ≥ 25 kg/m^2) and obesity (BMI ≥ 30 kg/m^2) was respectively 60.1% and 25.0%. Half of all DM cases in the population could be attributed to excess weight.

Conclusion: We found a high prevalence of DM and pre-diabetes in a rapidly developing country in the African region. The strong association between overweight and DM emphasizes the importance of weight control measures to reduce the incidence of DM in the population. High rates of diabetic persons not aware of having DM in the population and insufficient cardiometabolic control among persons treated for DM stress the need for intensifying health care for diabetes.

Background

It is estimated that diabetes mellitus (DM) accounts currently for 5.2% of all deaths worldwide [1]. The number of people with DM is expected to double from 175 million in 2000 to 353 million in 2030 [2]. The largest increase is expected to occur in developing countries, with 305 million individuals likely to have DM by 2030 [2].

The prevalence of DM in adults varies markedly between different populations, e.g. 2.6% in Nigeria [3], 18% in Mauritius [4], and more than 50% in Pima Indians in the U.S. [5]. These differences have been related to unfavorable trends in factors such as overweight and sedentary habits, as demonstrated in longitudinal, ecological and migration studies [4-6] and to interactions between environmental and genetic factors when individuals become exposed to an obesogenic environment [4,7].

In developing countries, the prevalence of diabetes is markedly higher in urban than rural areas (e.g. 2.0% vs. 0.8% in Cameroon in 1999 or 14% vs. 5% in Egypt in 1995) [8,9]. There is also a gradient across socio-economic development stages, e.g. a prevalence of DM in African individuals of 2% in Cameroon, 9% in Jamaica, 11% in Trinidad and Tobago and 15% in Manchester [8,10], which further emphasizes the role of environment factors in populations of same genetic origin. However, data on the prevalence, awareness and control of DM remain limited in the African region.

Based on a population-based survey, we assessed the prevalence, awareness and control of diabetes (DM) in the Republic of Seychelles, a rapidly developing country in the African region. We also examined the relationship between body mass index, fasting serum insulin and DM and the proportion of all diabetic persons in the population that could be attributed to excess body weight.

Methods

The Republic of Seychelles consists of over 100 islands located in the Indian Ocean about 2000 km east of Kenya and 2000 km north of Mauritius, in the African region. Approximately 90% of the population of Seychelles lives on the main island (Mahé) and most of the remaining population resides on two nearby islands (Praslin and La Digue). Although intermarriage has blurred racial differences in many Seychellois, it can be considered that approximately two thirds of the population is of predominantly African descent, 15% is of predominantly Caucasian, Indian or Chinese descent, and a fifth is mixed between these various groups. The population of Seychelles can be considered as fairly urbanized in view of the high density of the population and the facts that a large proportion of the population commutes to the capital for work and three quarters of workers are employed in services [11,12]. The national gross domestic product per capita, in real terms, rose from US$ 2927 in 1980 to US$ 5239 in 2004 [13], reflecting booming tourism and industrial fishing industries. In Seychelles, cardiovascular disease and AIDS currently account for 38% and 1% of all deaths, respectively [11]. Health care (inclusive medications) is provided free of charge to all inhabitants. The disease burden related to diabetes is significant in Seychelles (e.g. a majority of lower limb amputations and a third of all persons under hemodialysis are related to diabetes), although the precise diabetes-related disease burden has not been systematically determined yet.

A population-based examination survey for cardiovascular risk factors was conducted in 2004 under the auspices of the Ministry of Health of the Republic of Seychelles. The sampling frame consisted of a sex- and age- stratified random sample of the population aged 25–64. Eligible individuals were selected from a computerized database derived from population censuses (last in 2002) thereafter regularly updated by civil status authorities. Eligible participants were invited to participate through a personal letter requesting them to attend designated survey centers on a particular date, fasting, between 7:30 and 11:00 am and informing them that snacks would be provided on the study center. Participants were free to participate and gave informed written consent. The survey was approved by the research and ethical board of the Ministry of Health of the Republic of Seychelles.

A structured questionnaire was administered by trained survey officers. The questions assessed, among other items, if participants were "ever told by a doctor that they had DM" and if they "were currently under treatment for DM". If treatment was reported, participants were considered to have "known" DM. Family history of DM was defined for participants who reported DM among a first degree parents or siblings. Weight was measured with electronic scales to 0.2 kg precision (Seca, Hamburg, Germany) and height was measured with fixed stadiometers to 0.5 cm precision (Seca). BMI was calculated as weight divided by height squared (kg/m^2). Blood pressure (BP) refers to the average of the second and third of three measurements (mercury sphygmomanometer, cuff size adjusted to arm circumference).

Venous blood glucose was measured with a Cholestec LDX, a point-of-care analyzer which is a reliable alternative to conventional laboratory devices [14]. The Cholestec instrument separates blood cells from plasma and measurements are therefore made on plasma. If glucose was ≥ 5.6 mmol/l, another measurement was carried out a few minutes later on capillary blood (Bayer, Ascentia Elite [15]) and the mean of both readings was used. Of note, the Ascentia Elite automatically adjusts reading to

plasma values. The difference between the first measurement (Cholestec) and the second measurement (Ascentia Elite) was as small as -0.15 mmol/l. Individuals who had FBG ≥ 5.6 mmol/l and <7.0 mmol/l and were not aware of having DM were submitted to an oral glucose tolerance test (OGTT) using 75 g glucose dissolved in 300 ml water and capillary glucose (Ascentia Elite) was measured 120 minutes later (2hBG). Glycated hemoglobin (HbA1c) was measured in known or new cases of DM using a point-of-care analyzer (DCA 2000, Bayer). The DCA 2000 has been recommended for measurement of HbA1c outside of the laboratory [16].

Categories of impaired glucose regulation were based on the 2004 criteria of the American Diabetes Association [17]. DM was defined as plasma FBG ≥ 7.0 mmol/l, 2hBG ≥ 11.1 mmol/l or current history of antidiabetic medication. IFG refers to FBG of 5.6–6.9 mmol/l. IGT was defined as FBG <7.0 mmol/l and 2hBG of 7.8–11.0 mmol/l. Normal glucose tolerance (NGT) was defined as 2hBG <7.8 mmol/l. Normal fasting glucose (NFG) refers to FBG values <5.6 mmol/l. Since subjects with NFG were not tested for 2-hour glucose, we cannot determine DM based on 2hBG ≥ 11.1 mmol/l and NFG.

Serum was obtained within 2 hours of blood collection and immediately frozen to -20°C. Fasting serum insulin (FSI) was measured at the University of Lausanne using commercial RIA kits (LINCO Research Inc, Missouri, USA). HOMA-IR (homeostasis model assessment of insulin resistance) was calculated as [FSI (μU/ml) × FBG (mmol/l)]/22.5 [18]. Blood lipids were measured using standard methods (Hitachi 917 instrument and Roche reagents) and low-density lipoprotein cholesterol (LDL-C) calculated with the Friedewald formula.

Overall estimates in the population aged 25–64 were standardized to the new age distribution of the World Health Organization [19], using weighted "svy" commands in Stata. Differences were tested with the chi-square test and the t-test, respectively. For medians interquartile ranges were calculated. Increases in mean FSI across categories of impaired glucose regulation and BMI categories were tested with the Cuzik trend test. The association between DM and body mass index categories was analyzed with logistic regression adjusted for age and sex and weighted for the age-stratified sampling frame ("svylogit"). We used a model aggregating men and women since the interaction of BMI with sex was not significant. We calculated the proportions of all DM cases in the population that could be attributable to overweight and obesity (population attributable fraction, PAF) using the weighted "aflogit" command in Stata. PAF is conceptually computed as P(RR-1)/[1+P(RR-1)], where P is the prevalence of excess weight in the population [20] and RR is the risk ratio of the exposure (excess weight) on the outcome (DM). RR was estimated with the adjusted odds ratio (OR) derived from the multivariate logistic regression models and confidence intervals were based on asymptotic approximation [20]. Analyses were performed with Stata 8.2 and p values less than 0.05 were considered significant.

Results

1255 out of 1565 (80.3%) eligible individuals participated. Age-standardized mean values and prevalence rates of selected characteristics are presented in Table 1. Mean and median BMI and prevalence of overweight and obesity were higher in women than in men ($p < 0.001$). Mean FBG was similar in men and in women. Mean and median FSI were higher in women than in men ($p < 0.001$).

Table 2 shows the prevalence of different categories of impaired glucose regulation. The prevalence of DM was particularly high in the oldest age group (55–64) in both sexes. The prevalence of IFG was higher in men than in women ($p < 0.001$). Since the measurement of FBG alone may leave some DM persons undetected, an OGTT was performed in individuals with FBG between 5.6–6.9 mmol/l. Using OGTT results for the diagnosis of DM (i.e. 2hBG ≥ 11.1 mmol/l) in addition to other criteria for DM, the overall prevalence of DM increased by 2.1% (absolute difference) or 22% (relative difference). The prevalence of IGT did not differ significantly ($p > 0.05$) between genders. Of note, the prevalence of combined IFG/IGT is necessarily equal to the prevalence of IFG in our study since OGTT was performed in all subjects with IFG (i.e. FBG between 5.6 and 6.9 mmol/l). Based on all three criteria for DM, the prevalence of DM standardized for the actual population of Seychelles in 2004 (i.e. not adjusted to the WHO standard population) was 10.2% overall (95%CI: 8.6–11.9); men: 10.2% (7.7–12.7), women: 10.3 (8.0–12.6). The slightly lower prevalence using actual age distribution in Seychelles vs. the WHO age distribution reflects that the proportion of young vs. old people is slightly larger in the actual population of Seychelles than in the WHO standard age distribution.

Table 3 shows the odds ratios relating excess body weight to DM and the proportions of DM cases in the population that could be attributable to overweight and obesity, by sex and overall. Estimates are standardized to the WHO age distribution and regression models are also adjusted for age. The prevalence of overweight and obesity was higher in women than in men ($p < 0.001$). DM was strongly associated with excess body weight (e.g. OR in both men and women: 2.6 for BMI more vs. less than 25 kg/m^2). The OR of DM associated with overweight and obesity appearing in the table were virtually unchanged if underweight (BMI <18.5 kg/m^2, less than 6%) was also

Table 1: Age-standardized means and medians of selected characteristics by sex

		Men		Women		All	
n		568		687		1255	
Age (years)							
25–34†	%	22.2		21.7		21.9	
35–44†	%	23.6		25.6		24.7	
45–54†	%	27.8		26.3		27.0	
55–64†	%	26.4		26.4		26.4	
Mean age†	years	45.3	(0.5)	45.1	(0.4)	45.2	(0.3)
Mean age	years	42.1	(0.5)	41.9	(0.4)	42.0	(0.3)
Mean body mass index	kg/m^2	25.5	(0.2)	28.3	(0.2)	26.9	(0.2)
Median body mass index	kg/m^2	25.2	(6.3)	27.8	(8.5)	26.4	(7.4)
Prevalence underweight	%	4.6	(0.9)	3.3	(0.7)	4.0	(0.6)
Prevalence excess weight	%	51.9	(2.1)	68.3	(1.9)	60.1	(1.4)
Prevalence obesity	%	15.0	(1.5)	35.1	(1.8)	25.0	(1.2)
Mean fasting blood glucose	mmol/l	6.0	(0.1)	5.7	(0.1)	5.9	(0.1)
Median fasting blood glucose	mmol/l	5.5	(0.8)	5.3	(0.7)	5.4	(0.8)
Mean fasting serum insulin	pmol/l	81.0	(3.1)	96.6	(2.8)	88.8	(2.1)
Median fasting serum insulin	pmol/l	63.0	(48.6)	76.2	(55.2)	69.6	(52.2)
Mean HOMA-IR		4.1	(0.2)	4.5	(0.2)	4.3	(0.1)
Median HOMA-IR		2.7	(2.4)	3.1	(2.7)	2.9	(2.5)

Standard errors and inter-quartile ranges are indicated between brackets for means and medians, respectively. Except for age, all estimates are standardized for age [19].
Underweight: BMI <18.5 km/m^2;excess weight: BMI ≥ 25 km/m^2; obesity: BMI ≥ 30 kg/m^2.
HOMA-IR: homeostasis model assessment of insulin resistance.
† Crude estimates. Other estimates are standardized for age.

Table 2: Prevalence of diabetes, impaired fasting glucose and impaired glucose tolerance (percent and 95% confidence interval) and proportion of diabetic persons aware of having diabetes

	Men				Women				25–64		
	25–34	35–44	45–54	55–64	25–34	35–44	45–54	55–64	Men	Women	All
n	126	134	158	150	149	176	181	181	568	687	1255
Diabetes (DM)											
DM (2 criteria)	0.8 (0–2.3)	9.7 (4.7–15)	12.7 (7.5–18)	22.0 (15–29)	2.0 (0.2–2.3)	4.6 (1.5–7.6)	11.6 (6.9–16)	26.5 (20–33)	9.6 (7.4–12)	9.1 (7.2–11)	9.4 (7.9–11)
DM (3 criteria)	0.8 (0–2.3)	9.7 (4.7–15)	14.6 (9.1–20)	27.3 (20–35)	3.4 (0.5–2.9)	6.3 (2.7–9.8)	14.9 (9.7–20)	34.3 (27–41)	11.0 (8.7–13)	12.1 (9.9–14)	11.5 (9.9–13)
Impaired fasting glucose											
IFG	16.0 (9.6–22)	35.1 (27–43)	42.4 (35–50)	32.9 (25–40)	5.4 (1.7–3.6)	13.6 (8.6–19)	26.5 (20–33)	37.0 (30–44)	30.4 (27–34)	18.0 (15–21)	24.2 (22–26)
Glucose tolerance											
NGT (2hBG <7.8)	12.8 (6.9–19)	23.9 (16–31)	21.5 (15–28)	10.7 (5.8–16)	2.7 (0.1–2.6)	2.3 (0.1–4.5)	8.8 (4.7–13)	12.7 (7.8–18)	17.6 (14–21)	5.7 (4.1–7.3)	11.6 (9.8–14)
IGT (2hBG 7.8–11.0)	3.2 (0.1–6.3)	10.5 (5.3–16)	18.4 (12–24)	17.5 (11–24)	2.0 (0–2.3)	9.7 (5.3–14)	14.4 (9.2–20)	17.1 (12–23)	11.2 (8.7–14)	9.6 (7.5–12)	10.4 (8.8–12)
DM (2hBG ≥ 11.1)	0.0	0.8 (0–2.2)	2.5 (0.1–5.0)	4.7 (1.3–8.1)	0.7 (0–1.3)	1.7 (0–3.6)	3.3 (0.7–5.9)	7.2 (3.4–11)	1.6 (0.7–2.5)	2.7 (1.6–3.8)	2.1 (1.4–2.9)
Aware of DM (among DM)											
	0.0	30.8 (4.7–56)	45.0 (23–67)	60.6 (44–77)	0.0	50.0 (13–87)	61.9 (41–83)	64.6 (51–78)	47.1 (35–59)	61.5 (50–73)	53.9 (46–62)

FBG: fasting blood glucose; IFG: impaired fasting glucose; 2hBG: blood glucose 2 hours after oral tolerance test; NGT: normal glucose tolerance; IGT: impaired glucose tolerance; DM: diabetes mellitus.
DM (2 criteria): FBG ≥ 7.0 mmol/l or history of treatment for DM.
DM (3 criteria): FBG ≥ 7.0 mmol/l, history of treatment for DM, or 2hBG ≥ 11.1 mmol/l.
Oral glucose tolerance test was administered to all individuals with FBG of 5.6–6.9 mmol/l without previous history of diabetes.
Overall prevalence estimates are standardized for age [19].

factored in the analysis (the reference BMI category being then 18.5–24.9 kg/m^2). The proportion of all DM cases in the entire population that could be attributed to excess weight was 49% (95%: 35%–61%). By using lower cut off values to define the reference BMI category -as used in other studies [21]-, the proportions of all cases of DM in the population that are attributable to excess weight increased to 58% (95% CI: 56%–60%) for a BMI cut off set at ≥ 24 kg/m^2 and to 73% (71%–74%) for a BMI cut off set at ≥23 kg/m^2.

In a separate model, the odds for having DM were 2.4 (1.7–3.5) times higher in persons with family history of DM compared to those without it. This OR for family history of DM was virtually identical whether BMI was included in multivariate analysis or not and whether models were run in all participants, men or women. Adjusting for age, sex and BMI, the proportion of all DM cases in the population that could be attributable to family history of DM was 25% (14–35%).

Figure 1 shows that mean FSI increased gradually across both categories of BMI and categories of impairment of glucose metabolism (i.e. NFG, IFG, IGT, DM). In separate analyses adjusted for age and sex (analyses not shown), the same relationships were found between FSI and both categories of impaired glucose regulation and BMI categories (p for trend < 0.001 for both). In these analyses, DM patients who were on insulin treatment (n = 10) were excluded. Analyses with HOMA-IR instead of FSI showed same patterns of association with categories of BMI and glucose metabolism impairment.

Figure 2 shows, among all persons reporting current antidiabetic treatment (n = 80), the proportions who achieved different levels of blood glucose (based on HbA1c), BP and LDL-cholesterol. Less than a quarter of diabetic persons under treatment achieved recommended treatment targets for any of these three considered cardiometabolic conditions, i.e. HbA1c <7, BP <130/80 mmHg, and LDL-cholesterol <2.6 mmol/l. Less than 50% of treated patients achieved levels HbA1c <8, BP <140/90 mmHg and LDL-cholesterol <3.5 mmol/l. Almost 40% had HbA1c ≥ 10 and approximately 20% had BP ≥ 160/100 or LDL cholesterol ≥ 5 mmol/l. The prevalence of high blood pressure and hypercholesterolemia in this population were published earlier [22].

Discussion

We found a high prevalence of DM in a rapidly developing country in the African region, a substantial proportion of persons unaware of DM in the population, limited cardiometabolic control among treated diabetic persons, and a strong association of DM with excess weight. These findings in Seychelles add to the few population-based data on DM available in the African region and may be representative of other countries experiencing rapid socio-economic development and concurrent lifestyle changes.

The prevalence of DM is higher in Seychelles than in predominantly rural African regions such as Nigeria [3], Cameroon [23] or Tanzania [24] but as high as in urban settings in South Africa [25] and Egypt [9]. The prevalence of DM in Seychelles is however lower than in the island of Mauritius [4] despite larger BMI in the population of Seychelles than in Mauritius. The difference between these two neighbor islands of similar economic development may partly relate to their different ethnic composition (predominantly African in Seychelles and predominantly Indian in Mauritius) since Indian descent is a known risk factor for DM [4,26]. Compared to non African countries, the prevalence of DM in the Seychelles is similar to estimates in urban Saudi Arabia [27], several regions of Europe [28], the United States [29] and urban India [6] but higher than in Mongolia [30], Bangladesh [31], the

Table 3: Relationship between categories of body mass index (BMI) and diabetes and proportion of diabetic persons in the entire population that is attributable to overweight and obesity (95% confidence intervals in brackets)

	Men			Women			All		
	Prevalence (%)	Odds ratio*	PAF (%)	Prevalence (%)	Odds ratio*	PAF (%)	Prevalence (%)	Odds ratio*	PAF (%)
Overweight (BMI: 25–29 kg/m^2)	37 (33–41)	2.1 (1.9–2.2)	26 (16–36)	33 (30–37)	2.6 (2.3–3.0)	20 (12–26)	35 (32–38)	3.5 (3.2–3.8)	23 (14–31)
Obesity (BMI ≥ 30 kg/m^2)	15 (12–18)	2.6 (2.4–2.9)	17 (11–23)	35 (32–29)	4.5 (4.0–5.1)	36 (25–45)	25 (23–28)	3.3 (2.0–5.5)	26 (15–36)
Overweight or obesity (BMI ≥ 25 kg/m^2)	52 (48–56)	2.2 (2.1–2.4)	43 (29–55)	68 (64–72)	3.6 (3.2–4.1)	56 (41–67)	60 (57–63)	2.6 (2.4–2.8)	49 (35–61)

All models are adjusted for age.
Estimates are standardized for age [19].
BMI: body mass index.
PAF: population attributable fraction.
* reference category: BMI <25 kg/m^2.

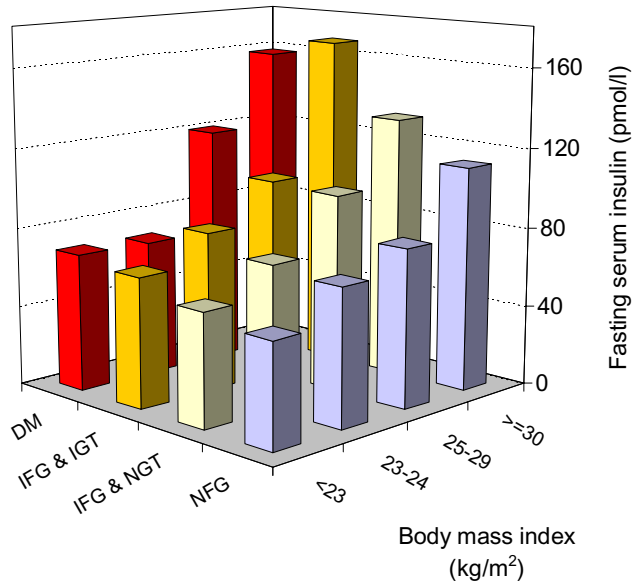

Figure 1
Mean fasting serum insulin concentration by categories of body mass index (BMI) and categories of glucose metabolism impairment. NFG: normal fasting glucose; IFG: impaired fasting glucose; NGT: normal glucose tolerance; IGT: impaired glucose tolerance; DM: diabetes mellitus (excluding patients on insulin treatment).

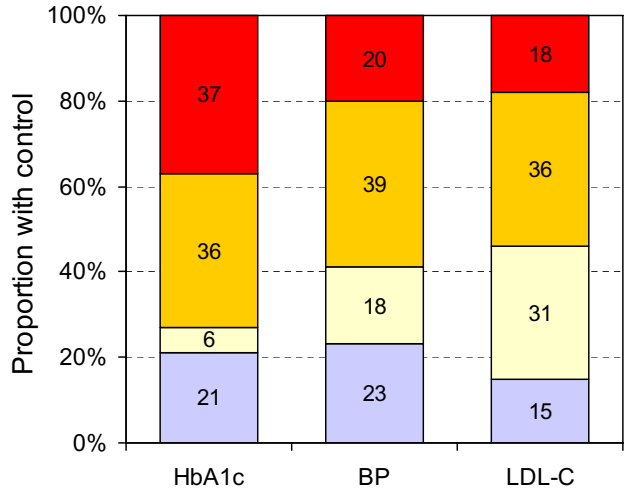

Figure 2
Level of control of blood glucose (HbA1c: glycated-hemoglobin), blood pressure (BP) and low-density lipoprotein cholesterol (LDL-C) in all participants receiving hypoglycemic treatment (n = 80). Cut off values for control categories are <7.0, 7.0–7.9, 8–9.9, ≥ 10 for HbA1c; <130/80; 130-9/80-9; 140-59/90–99, ≥ 160/100 mmHg for BP; and <2.6, 2.6–3.4, 3.5–4.9, ≥ 5 mmol/l for LDL-cholesterol. Blue: within recommended treatment targets.

Philippines [32], Spain [33], Australia [34], Turkey [35] and rural Saudi Arabia [27].

A few factors in our study may tend to over or underestimate the true prevalence of DM in the population. Possible biases for underestimation are several. First we did not include individuals older than 64 years, an age group in which the prevalence of DM is likely to be particularly high [4,29,36,37]. Second, we did not perform an OGTT in individuals with FBG <5.6 mmol/l and we could have missed a few DM cases in persons with normal fasting glucose but pathologically high post meal levels. The number of such cases is however expected to be small [38]. Third, underestimation might also have occurred if non participation was related to diabetes-related diseases (e.g. leg wounds, renal failure, stroke, etc). On the other hand, overestimation may have occurred if some persons with elevated FBG were not fasting. This bias was minimized as persons who reported not to be fasting were invited to attend the survey on another day. Also, elevated fasting blood glucose was not repeated on a separate day. Overall, it is possible that factors that over or underestimate the prevalence of DM might balance each other and our prevalence estimates may be close to the true prevalence of DM in the adult population.

The high prevalence of IFG and/or IGT suggests that the DM epidemic has not yet plateaued in Seychelles. Indeed, IGT [39] and IFG [40] are strong predictors of DM [41] and these pre-diabetes conditions may occur in up to 60% of individuals several years before DM develops [42]. It has been reported that in the USA, where the prevalence of IFG and IGT is respectively 26% and 15%, approximately 25% of individuals with IFG/IGT will progress to diabetes, 50% will remain in their abnormal glycemic state, and 25% will revert to normal glucose tolerance (NGT) over a period of 3 to 5 years [43]. Hence, the substantially high proportion of pre-diabetes in Seychelles predicts a further increase in the prevalence of DM over the next few years, consistent with projections in other African countries in epidemiological transition [23,26,36].

While the proportion of all diabetes cases in the population who are aware of having DM ("aware") is approximately 50%, similar low proportions (about 50%) are also typically found in middle- or high-income countries such as Egypt [9], Saudi Arabia [27], Spain [33] and the U.S. [44]. Such low figures emphasize the difficulty to identify a disease (DM) that is most often silent for many years after onset.

We found that a large proportion of diabetic persons under anti-diabetic treatment had levels of blood sugar (as assessed by HbA1c), blood pressure, and LDL-cholesterol above the recommended therapeutic targets. Limited clinical control of DM is also found in high income western countries such as the USA [45]. In the USA, only 37% of DM patients had HbA1c levels <7.0%, only 35.8% achieved target blood pressure (<130/80 mmHg) and 51.8% of DM patients had hypercholesterolemia [45]. This further illustrates the great difficulty in achieving and sustaining good control of blood glucose, blood pressure and blood lipids in chronic diseases such as DM. In addition, limited cardiometabolic control is known to be even more difficult to achieve in DM patients who are overweight -a frequent occurrence- because of underlying insulin resistance [45,46].

DM was associated strongly with overweight, independent of age, sex and family history of DM. This relationship has been found consistently in other populations [27,32,33,35,47] and, for example, 90% of new DM cases among both African and white Americans had BMI ≥ 23 kg/m^2 [21]. In our study, half of all DM cases could be attributed to overweight or obesity. This proportion rose to 73% if normal weight was considered for BMI <23 kg/m^2 instead of BMI <25 kg/m^2. In a Taiwanese cohort as much as 71% of DM cases were attributable to a BMI ≥ 25 kg/m^2[48].

It is important to attempt to determine if the prevalence rates of DM and overweight (i.e. "diabesity") has increased over time in order to anticipate epidemiological trends and inform health care policy. The prevalence of DM was assessed for the first time in 1989 in the population of Seychelles [49] and it was found that 3.4% of men and 4.6% of women had DM. Using the same criteria in 1989 and 2004 (i.e. known diabetes and/or elevated fasting blood glucose) and the same age standardization (new WHO age distribution [20]) the prevalence of DM significantly increased between the two years in men (from 6.2% to 9.6%) and in women (from 6.1% to 9.1%). Since the survey methods were not identical in 1989 and 2004 (e.g. glucometers), there is a degree of uncertainty in these trend estimates. However, a true increase in the prevalence of DM over time is consistent with the large increase in obesity in men (from 4.3% to 15.0%) and women (from 27.9% to 35.1%). Increasing prevalence of overweight/obesity in the population in the interval is likely related to increasingly sedentary behaviors and larger caloric intake. Currently, more than 75% of workers are employed in services (vs. only 20% in industry and 5% in agriculture) [11,12]. The number of both private cars and passengers transported by public buses has doubled in the past 10 years (figures from the Licensing Authority and the Seychelles Public Transport Company, respectively). On the other hand, food balance sheets indicate that calorie availability per capita has increased substantially in Seychelles, e.g. from 1800 kcal in 1965, 2300 kcal in the late 1980s, and above 2400 kcal in the early 2000s [50]. The proportion of carbohydrates has decreased over time (74% of total calories in 1965 and 55% in 2000) while the proportion of fats has increased (16% in 1965 and 32% in 2000) [50]. The production of carbonated soft drinks by the main local manufacturer has tripled in the past 25 years (figures from Seychelles Breweries Ltd).

Conclusion

The prevalence of DM in Seychelles has reached or exceeded levels typically found in several middle- or high-income countries. The strong association between DM and excess body weight emphasizes the importance of weight control interventions at a population level as a cornerstone strategy to curb the "diabesity" epidemic [51]. From a clinical perspective, the substantial proportion of persons unaware of having DM calls for improved early detection of diabetic persons. The high proportion of treated diabetic persons with insufficient cardiometabolic control stresses the need for intensifying clinical care to diabetic patients in order to minimize complications [52].

Abbreviations

DM: diabetes mellitus; FBG: fasting blood glucose; IFG: impaired fasting glucose; IGT: impaired glucose tolerance; HOMA: homeostasis model assessment of insulin resistance; 2-hour OGTT: oral glucose tolerance test; 2hBG: 2-hour postload blood glucose; BP: blood pressure; LDL-C: low-density lipoprotein cholesterol; HbA1c: glycated haemoglobin; PAF: population attributable fraction.

Competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

DF lead the analysis of the data and the write up of the manuscript. JW coordinated several aspects of the survey and reviewed the manuscript. LT performed insulin assays and reviewed the manuscript. ER assisted in the interpretation of data and reviewed the manuscript. PB lead the organization of the survey, assisted with the analysis and interpretation of the data and with the write up of the manuscript. All authors read and approved the final manuscript.

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Diabetes and pre-diabetes are associated with cardiovascular risk factors and carotid/femoral intima-media thickness independently of markers of insulin resistance and adiposity

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Abstract

Background: Impaired glucose regulation (IGR) is associated with detrimental cardiovascular outcomes such as cardiovascular disease risk factors (CVD risk factors) or intima-media thickness (IMT). Our aim was to examine whether these associations are mediated by body mass index (BMI), waist circumference (waist) or fasting serum insulin (insulin) in a population in the African region.

Methods: Major CVD risk factors (systolic blood pressure, smoking, LDL-cholesterol, HDL-cholesterol,) were measured in a random sample of adults aged 25–64 in the Seychelles (n = 1255, participation rate: 80.2%).

According to the criteria of the American Diabetes Association, IGR was divided in four ordered categories: 1) normal fasting glucose (NFG), 2) impaired fasting glucose (IFG) and normal glucose tolerance (IFG/NGT), 3) IFG and impaired glucose tolerance (IFG/IGT), and 4) diabetes mellitus (DM). Carotid and femoral IMT was assessed by ultrasound (n = 496).

Results: Age-adjusted levels of the major CVD risk factors worsened gradually across IGR categories (NFG < IFG/NGT < IFG/IGT < DM), particularly HDL-cholesterol and blood pressure (p for trend < 0.001). These relationships were marginally attenuated upon further adjustment for waist, BMI or insulin (whether considered alone or combined) and most of these relationships remained significant. With regards to IMT, the association was null with IFG/NGT, weak with IFG/IGT and stronger with DM (all more markedly at femoral than carotid levels). The associations between IMT and IFG/IGT or DM (adjusted by age and major CVD risk factors) decreased only marginally upon further adjustment for BMI, waist or insulin. Further adjustment for family history of diabetes did not alter the results.

Conclusion: We found graded relationships between IGR categories and both major CVD risk factors and carotid/femoral IMT. These relationships were only partly accounted for by BMI, waist and insulin. This suggests that increased CVD-risk associated with IGR is also mediated by factors other than the considered markers of adiposity and insulin resistance. The results also imply that IGR and associated major CVD risk factors should be systematically screened and appropriately managed.

Background

Worldwide, the number of persons with diabetes mellitus (DM) is expected to double in the next 25 years and to affect more than 350 million individuals by 2030 [1]. Accordingly, there is a growing interest in identifying individuals in stages preceding overt DM in order to potentially prevent the occurrence of DM and associated complications. The majority of complications of DM are related to cardiovascular disease (CVD) and it is therefore important to assess whether pre-diabetes stages are also associated with detrimental vascular outcomes [2].

Pre-diabetes has been first described by the World Health Organization in 1980 as impaired glucose tolerance (IGT) [3]. In order to avoid the time-consuming and somewhat cumbersome measurement of 2-hour postload glucose concentrations (2hBG), the American Diabetes Association (ADA) proposed in 1997 to identify pre-diabetes as impaired fasting glucose (IFG), which relies on one fasting measurement only. In 2004, the ADA lowered the cut-off point for IFG from 6.1 to 5.6 mmol/l [4]. It has been demonstrated that both IFG and IGT are risk factors for the subsequent development of both DM [5,6] and CVD [2,5,7]. However, the respective prognostic values of IFG and IGT to predict DM and CVD risk are still controversial [5,8-11].

Because they measure different aspects of glucose metabolism, IFG and IGT have different underlying physiological and clinical significance with respect to insulin sensitivity and secretion [12-14]. Subsequently, these two categories of impairment of glucose regulation (IGR) may differently relate to CVD risk factors. However, both IFG and IGT are strongly associated with excess body weight, which in turn is associated with insulin resistance. Hence, the relationship between IGR categories and CVD risk factors or CVD outcomes may be mediated by such markers of adiposity or insulin resistance.

Intima-media thickness (IMT) of peripheral arteries (particularly carotid IMT) can be regarded as a cardiovascular risk factor since it is an independent predictor of CVD morbidity and mortality [15]. However, IMT also measures organic vascular changes -atherosclerosis- [16] and it is therefore also increasingly used as a proxy of CVD outcomes [17,18].

In this study we compared the association between IGR categories (IFG, IGT and DM) and both 1) major CVD risk factors (blood pressure, smoking, LDL-cholesterol, HDL-cholesterol) and 2) IMT (carotid and femoral IMT). We hypothesized that these associations would be substantially explained by markers of adiposity and fasting serum insulin concentration, hence that these relationships would not remain significant upon adjustment for these markers. We examined these relationships in a sample representative of the general population of the Seychelles, a rapidly developing country in health transition in the African region. The distribution of the main cardiovascular risk factors, pre-diabetes and diabetes in this population has been described previously [19-21].

Methods

Population

The Republic of Seychelles consists of over 100 islands located in the Indian Ocean about 2000 km east of Kenya and 2000 km north of Mauritius, in the African region. Approximately 90% of the population lives on the main island (Mahé) and most of the remaining population resides on two nearby islands. Although intermarriage has blurred racial differences in many Seychellois, it can be considered that approximately two thirds of the population is of predominantly African descent, 15% is of predominantly Caucasian, Indian or Chinese descent, and a fifth is mixed between these groups. The population can be considered as fairly urbanized in view of the high density of the population and the facts that a large proportion of the population commutes to the capital for work and three quarters of workers are employed in services [22,23]. The national gross domestic product per capita, in real terms, rose from US$ 2927 in 1980 to US$ 5239 in 2004 [24], reflecting booming tourism and fishing industries. All deaths are registered in Seychelles and vital statistics indicate a life expectancy at birth of 69 years in men and 76 years in women. In Seychelles, cardiovascular disease and AIDS currently account for 38% and 1% of all deaths, respectively [25].

Participants

A population-based survey for cardiovascular risk factors was conducted in 2004 under the auspices of the Ministry of Health of the Republic of Seychelles. The sampling frame consisted of a sex- and age- stratified random sample of the population aged 25–64. Eligible individuals were selected from a computerized database of the entire population derived from the population census carried out in 2002, thereafter updated by civil authorities. Participants were invited to participate through a personal letter requesting them to attend a survey center on a specified day, fasting, between 7:30 and 11:00 am. Individuals were free to participate and gave written informed consent. The survey was approved by the Ministry of Health following technical and ethical reviews.

Categories of impaired glucose regulation

Venous blood glucose (FBG) was measured with a Cholestec LDX, a point-of-care analyzer which is a reliable alternative to conventional laboratory devices [26]. The Cholestec instrument separates blood cells from plasma and measurements are therefore made on plasma. If glu-

cose was ≥ 5.6 mmol/l, another measurement was carried out a few minutes later on capillary blood (Bayer, Ascentia Elite [27]) and the mean of both readings was used. Of note, the Ascentia Elite automatically adjusts its glucose readings to plasma values. The difference between the first FBG measurement (Cholestec) and the second FBG measurement (Ascentia Elite) was as small as -0.15 mmol/l. Individuals who were not aware of DM and with FBG ≥ 5.6 mmol/l and < 7 mmol/l were submitted to an oral glucose tolerance test (OGTT) using a meal of 75 g glucose dissolved in 300 ml water and capillary glucose (Ascentia Elite) was measured 120 min later (2hBG). Categories of impaired glucose regulation (IGR) were based on the new criteria of the ADA [4]. DM was defined as FBG ≥ 7.0 mmol/l, 2hBG ≥ 11.1 mmol/l, or a current history of antidiabetic medication. IFG refers to FBG of 5.6–6.9 mmol/l. IGT was defined as FBG < 7.0 mmol/l and 2hBG of 7.8–11.0 mmol/l. Normal glucose tolerance (NGT) was defined as 2hBG < 7.8 mmol/l. NFG refers to FBG values < 5.6 mmol/l.

Covariates

A structured questionnaire was administered to participants by trained survey officers. Family history of DM was defined as reported DM among a first degree parent or sibling. Weight was measured with an electronic scale at 0.2 kg precision (Seca, Hamburg, Germany), height with a fixed stadiometer at 0.5 cm precision (Seca). BMI was calculated as weight divided by height squared (kg/m^2). Waist circumference (waist) was measured at the level of the umbilicus in standing position, with individuals in light garments. Blood pressure (BP) refers to the average of the second and third of three measurements (mercury sphygmomanometer, cuff size adjusted to arm circumference). Smoking was defined as current smoking of at least 1 cigarette/day.

Serum was obtained within 2 hours of blood collection and immediately frozen to -20°C. Fasting serum insulin (insulin) was measured using commercial RIA kits (LINCO Research Inc, Missouri, USA). Blood lipids were measured using standard methods (Hitachi 917 instrument and Roche reagents). In this paper, we refer to CVD risk factors to designate major CVD risk factors, i.e. systolic blood pressure, smoking, LDL-cholesterol, and HDL-cholesterol. We used insulin as our primary marker of insulin resistance. We preferred insulin (to HOMA (homeostasis model assessment of insulin resistance) as the former is directly measured and the latter is calculated and includes a variable (fasting blood glucose) that is also used to define categories of IGR in our analyses.

Ultrasonography

High-resolution B-mode ultrasonography was conducted in all participants above 45 years seen during a 17-week period (n = 496) as well as in a randomly selected sample (18%, n = 57) of the participants aged 35–44 years. We restricted the investigation to this age range because older persons are more likely to have atherosclerosis. Carotid intima-media thickness is a well-established surrogate marker for atherosclerotic disease that is increasingly used in observational and interventional studies [28]. All the scans and image measurements were carried out by the same investigator (P.Y.) who was blinded to the risk factor status of the participants. We used a portable ultrasound system (General Electric LogiqBook) connected with a 6–10 MHz linear array transducer. The system was equipped with a software (M'ATH, ICN-metric, Paris, France) to perform semi-automatic measures of intima-media thickness (IMT) on frame [29]. IMT was measured on the far wall of both the right and left common carotid and femoral arteries over a length of 1 cm on a reference site located 2 cm below the bifurcation. The measurements on the left and right arteries were averaged to obtain a single mean value at carotid and femoral levels and all four measurements were averaged to obtain a combined value for all four arteries. The far wall was used because of higher reproducibility and possible overestimation of the IMT of the near wall [30]. To examine the reproducibility, we made a repeat investigation in 20 randomly selected participants within few week intervals. For carotid IMT, the coefficient of variation was 4.8%, which is similar to findings in other studies [31]. For femoral IMT, the coefficient of variation was 9.2%.

Statistical analysis

Prevalence estimates were standardized to the age distribution of the WHO [32]. We tested differences in means (± standard errors, SE) of CVD risk factors across categories of IRG with the chi-square test and the t-test, respectively. One-way analysis of variance was used to compare categorical variables between the four groups. Trends were calculated with the Stata-command "nptrend" (by Cuzick, 1985 and Altman, 1991). The associations between IFG/NGT, IFG/IGT and DM and risk factors were analyzed with multivariate linear regression. Models were adjusted for age, sex, BMI, waist, insulin, systolic BP, LDL-cholesterol, HDL-cholesterol and smoking status. We did not include triglyceride in the main multivariate analyses because: i) the independent role of triglycerides remains controversial; ii) triglyceride is generally not included as a major risk factor in CVD risk models; iii) triglyceride is strongly associated with resistance insulin and/or HDL cholesterol, as we have shown in the same data [20]. Analyses were performed with Stata 8.2 and P values < 0.05 were considered significant.

Results

1,255 persons (568 men and 568 women) participated out of 1,565 eligible individuals, a participation rate of

80.3%. About one third (33.5%) of participants had IFG/NGT (11.6%), IFG/IGT (10.4%) or DM (11.5%). However, while the prevalence did not differ by gender for DM (11.0% in men and 12.1% in women) and IFG/IGT (11.2% in men and 9.6% in women), the prevalence of IFG/NGT was higher in men than in women (17.6% vs. 5.7%, $p < 0.001$). The overall age-adjusted prevalence (± SE) of family history of diabetes was 30.0 ± 1.3% (men: 26.6 ± 1.9, women: 33.2 ± 1.8). The age-adjusted prevalence of family history of diabetes tended to increase across categories of NFG (26.6 ± 1.6%, men: 22.0 ± 2.3, women: 30.4 ± 2.2), IFG/NGT (28.9 ± 3.7, men: 23.3 ± 4.6, women: 27.5 ± 6.6), IFG/IGT (36.5 ± 4.0, men: 33.1 ± 5.5, women: 40.6 ± 5.6), and DM (44.0 ± 3.7, men: 40.5 ± 5.5, women: 47.5 ± 5.0).

Table 1 shows the distribution of anthropometric, clinical and ultrasound results by categories of IGR and by sex. The results are standardized for age using 10-year age categories. Mean BMI, waist and insulin were higher in women than in men in almost all IGR categories while mean triglycerides concentration was lower in women

Table 1: Distribution of selected clinical and metabolic factors across categories of impaired glucose regulation

Variable	NFG	IFG/NGT	IFG/IGT	DM	z-trend*
Men					
Clinical and metabolic data (n)	316	98	73	81	8.1
Age (years)	42.5 ± 0.6	45.1 ± 0.9	49.3 ± 1.1	52.7 ± 1.0	5.9
Body mass index (kg/m²)	24.6 ± 0.3	26.6 ± 0.4	26.6 ± 0.5	27.7 ± 0.6	8.2
Waist circumference (cm)	85.8 ± 0.6	92.5 ± 1.0	93.0 ± 1.4	97.5 ± 1.3	7.8
Serum fasting insulin (pmol/l)	10.8 ± 0.4	14.2 ± 0.8	19.8 ± 2.4	20.9 ± 2.1	6.9
Triglycerides (mmol/l)	0.97 ± 0.03	1.20 ± 0.07	1.56 ± 0.19	1.76 ± 0.17	2.5
LDL cholesterol (mmol/l)	3.42 ± 0.07	3.60 ± 0.13	3.83 ± 0.14	3.75 ± 0.17	-3.8
HDL cholesterol (mmol/l)	1.42 ± 0.03	1.29 ± 0.05	1.27 ± 0.06	1.20 ± 0.05	8.0
Systolic blood pressure (mmHg)	127 ± 0.9	132 ± 1.6	137 ± 1.9	145 ± 2.3	
Mean intima-media thickness (n)	104	46	48	50	2.4
Carotid (mm)	0.72 ± 0.01	0.71 ± 0.02	0.78 ± 0.03	0.75 ± 0.02	5.2
Femoral (mm)	0.93 ± 0.06	0.92 ± 0.09	1.20 ± 0.10	1.41 ± 0.13	4.9
Total (mm)	0.76 ± 0.02	0.82 ± 0.04	0.90 ± 0.03	1.04 ± 0.04	
Women					
Clinical and metabolic data (n)	460	47	77	103	
Age (years)	41.4 ± 0.5	52.1 ± 1.4	50.1 ± 1.0	54.4 ± 0.8	11.9
Body mass index (kg/m²)	27.1 ± 0.3	31.5 ± 0.9	31.8 ± 0.7	31.8 ± 0.5	8.4
Waist circumference (cm)	86.7 ± 0.6	100.1 ± 2.1	97.4 ± 1.4	101.5 ± 1.1	10.7
Serum fasting insulin (pmol/l)	13.8 ± 0.4	21.1 ± 1.9	19.4 ± 1.4	25.4 ± 2.1	8.4
Triglycerides (mmol/l)	0.82 ± 0.02	1.07 ± 0.08	1.10 ± 0.1	1.35 ± 0.08	8.8
LDL cholesterol (mmol/l)	3.44 ± 0.05	4.11 ± 0.19	3.81 ± 0.14	4.20 ± 0.14	5.8
HDL cholesterol (mmol/l)	1.40 ± 0.02	1.32 ± 0.05	1.28 ± 0.0	1.21 ± 0.04	-5.0
Systolic blood pressure (mmHg)	120 ± 0.7	137 ± 3.8	134 ± 2.1	139 ± 1.9	10.3
Mean intima-media thickness (n)	155	30	45	76	
Carotid (mm)	0.69 ± 0.01	0.75 ± 0.02	0.74 ± 0.04	0.84 ± 0.03	5.6
Femoral (mm)	0.71 ± 0.03	0.88 ± 0.09	0.84 ± 0.06	1.16 ± 0.07	6.5
Total (mm)	0.76 ± 0.02	0.82 ± 0.04	0.90 ± 0.03	1.04 ± 0.04	7.4
Overall					
Clinical and metabolic data (n)	776	145	150	184	
Age (years)	41.8 ± 0.4	47.4 ± 0.8	49.7 ± 0.7	53.7 ± 0.6	14.3
Body mass index (kg/m²)	26.0 ± 0.2	27.8 ± 0.4	29.0 ± 0.5	29.7 ± 0.4	9.5
Waist circumference (cm)	86.3 ± 0.4	94.3 ± 1.0	95.1 ± 1.0	99.5 ± 0.9	13.4
Serum fasting insulin (pmol/l)	12.5 ± 0.3	15.9 ± 0.8	19.6 ± 1.4	23.3 ± 1.5	11.1
Triglycerides (mmol/l)	0.89 ± 0.02	1.17 ± 0.05	1.35 ± 0.10	1.55 ± 0.09	11.2
LDL cholesterol (mmol/l)	3.43 ± 0.04	3.73 ± 0.11	3.82 ± 0.10	3.98 ± 0.11	5.8
HDL cholesterol (mmol/l)	1.41 ± 0.02	1.30 ± 0.04	1.27 ± 0.04	1.21 ± 0.03	-6.5
Systolic blood pressure (mmHg)	123 ± 0.6	134 ± 1.6	136 ± 1.4	142 ± 1.5	13.1
Mean intima-media thickness (n)	258	76	93	126	
Carotid (mm)	0.71 ± 0.01	0.72 ± 0.01	0.76 ± 0.02	0.80 ± 0.02	5.9
Femoral (mm)	0.81 ± 0.03	0.91 ± 0.07	1.04 ± 0.06	1.27 ± 0.07	8.3
Total (mm)	0.76 ± 0.02	0.82 ± 0.04	0.90 ± 0.03	1.04 ± 0.04	8.7

Values displayed are age-standardized means ± standard error.
*z-value for trend; all corresponding p-values < 0.01.

than in men in all IGR categories ($p < 0.05$). In contrast, no gender difference was found in HDL cholesterol, LDL cholesterol and BP across IGR categories. Interestingly, waist tended to increase more gradually along IGR categories than BMI resulting in a larger z-value for the trend test for waist than for BMI. Also, waist tended to increase between the IFG/IGT and DM categories while BMI did not. However, the proportional increase between NGT and DM categories was almost identical for BMI and waist for both men (~13%) and women (~17%). Tests for trend were significant for all variables (we show the z-values for the tests, i.e. the log of the p value, since all p-values are < 0.01). P values for all trends tended to be stronger in women than in men.

Table 2 shows the multivariate association between CVD risk factors and categories of IGR upon incremental adjustment for covariates related to adiposity (BMI and waist) and insulin resistance. All models are adjusted for sex and age, since age is strongly associated with IGR and the considered CVD risk factors (hence an important confounding factor). Incremental adjustment for BMI, waist and insulin allows disentangling confounding effects by these variables since adiposity and/or insulin resistance are known to be associated both with most of the considered CVD risk factors and IGR. Adjustment for BMI or waist reduced the magnitude of the regression coefficients between CVD risk factors and IGR only slightly. This attenuation effect tended to be larger with waist than BMI. The association between CVD risk factors and IGR categories was also slightly attenuated upon adjustment of insulin. Attenuation of the relationship between CVD risk factors and IGR was similar upon adjustment of BMI, waist or insulin. Concurrent adjustment for all three markers (BMI, waist and insulin) produced the smallest coefficients for all CVD risk factors in virtually all IGR categories. However, the attenuation of the relation between CVD risk factors and IGR categories was only marginally larger upon adjustment with all three adiposity/insulin resistance markers (BMI, waist, insulin) as compared to adjustment for any of these markers. This suggests that any of these three markers similarly reflected a common underlying mechanism.

The association between CVD risk factors and IGR categories increased generally fairly monotonically over increasing IGR categories, with as much an increase in the regression coefficients between the IFG/NGT and IFG/IGT

Table 2: Associations between categories of impaired glucose regulation and selected cardio-metabolic risk factors

	IFG/NGT			IFG/IGT			DM		
Adjustment in addition to age and sex	Coef.	SE	*p*	Coef.	SE	*p*	Coef.	SE	*p*
Triglycerides (mmol/l)									
None	0.19	0.07	0.01	0.39	0.07	0.00	0.59	0.07	0.00
BMI	0.14	0.07	0.05	0.33	0.07	0.00	0.52	0.07	0.00
Waist	0.10	0.07	ns	0.31	0.07	0.00	0.46	0.07	0.00
Insulin	0.15	0.07	0.03	0.29	0.07	0.00	0.48	0.08	0.00
BMI, Waist, Insulin	0.09	0.07	ns	0.25	0.07	0.00	0.41	0.08	0.00
Low-density lipoprotein cholesterol (mmol/l)									
None	0.26	0.11	0.02	0.29	0.12	0.02	0.39	0.12	0.00
BMI	0.19	0.11	0.09	0.21	0.12	0.07	0.30	0.12	0.01
Waist	0.15	0.11	ns	0.19	0.12	ns	0.24	0.12	0.04
Insulin	0.22	0.11	0.05	0.20	0.12	ns	0.23	0.12	0.06
BMI, Waist, Insulin	0.15	0.11	ns	0.15	0.12	ns	0.16	0.12	ns
High-density lipoprotein cholesterol (mmol/l)									
None	-0.14	0.04	0.00	-0.19	0.05	0.00	-0.27	0.05	0.00
BMI	-0.08	0.04	0.05	-0.12	0.04	0.01	-0.19	0.04	0.00
Waist	-0.06	0.04	ns	-0.11	0.04	0.02	-0.16	0.04	0.00
Insulin	-0.11	0.04	0.01	-0.14	0.05	0.00	-0.20	0.05	0.00
BMI, Waist, Insulin	-0.05	0.04	ns	-0.09	0.04	0.04	-0.14	0.05	0.00
Systolic blood pressure (mmHg)									
None	5.43	1.49	0.00	7.02	1.56	0.00	10.82	1.54	0.00
BMI	4.15	1.48	0.01	5.55	1.56	0.00	9.14	1.54	0.00
Waist	3.67	1.49	0.01	5.39	1.55	0.00	8.38	1.56	0.00
Insulin	4.57	1.50	0.00	5.72	1.59	0.00	9.48	1.64	0.00
BMI, Waist, Insulin	3.45	1.50	0.02	4.87	1.58	0.00	8.42	1.64	0.00

ns: $p \geq 0.10$.
NFG: normal fasting glucose; IFG: impaired fasting glucose; NGT: normal glucose tolerance; IGT: impaired glucose tolerance; DM: diabetes mellitus; BMI: body mass index; waist: waist circumference; insulin: serum fasting insulin.

categories as between the IFG/IGT and DM categories. All the considered CVD risk factors were associated with all three IGR categories, except for CRP and IFG/NGT. However, a few associations were no longer significant upon full adjustment for adiposity and insulin resistance markers (BMI, waist, insulin). For DM, the associations remained significant for all CVD risk factors except LDL-cholesterol. For IFG/IGT, the associations remained significant for all CVD risk factors except LDL-cholesterol and CRP. For IFG/NGT, the associations remained for triglyceride and systolic BP but not for LDL-cholesterol, HDL-cholesterol and CRP. The association of IGR with BP was similar whether based on diastolic or systolic BP (results not shown).

Family history of diabetes was associated with IFG/IGT and DM, but not with IFG/NGT. Adjustment for family history of DM in addition to age, sex, BMI, waist and insulin had virtually no impact on the associations (i.e. regression coefficients) between CVD risk factors and IGR categories (results not shown). This suggests that the relationships between CVD RF and IGR are not mediated by family history. In addition, IGR categories were associated, in adjusted models, with apo B and CRP but not with apo A1 and cystatin C (results not shown).

In Figure 1, we considered carotid/femoral IMT as a marker of atherosclerosis, hence as a CVD outcome. All associations between IFG/NGT and IMT were not statistically significant and are thus not shown. The analysis examines whether IGR categories predict IMT independently of age and CVD risk factors and whether an association, if any, would be sensitive to further adjustment of markers of adiposity or insulin resistance (BMI, waist, and insulin). The data show no association with IFG/NGT, a weak association with IFG/IGT, and a stronger association with DM. All these associations were stronger at femoral than carotid levels.

Adjustment for CVD risk factors attenuated the relationship between IGR categories and IMT, which suggests that a substantial part of the effect of IGR on IMT is mediated by BP, LDL-cholesterol, HDL-cholesterol and smoking status. Further adjustment for triglyceride, in addition to the considered major CVD risk factors, left the regression coefficients virtually unchanged (results not shown). Further adjustment for BMI, waist and insulin, whether considered separately or in combination, further reduced these relationships only marginally (as assessed by the further small relative decrease in the age and CVD risk factors adjusted regression coefficients). The associations between DM and either femoral IMT or total IMT (i.e. femoral + carotid) remained significant upon full adjustment for age, sex, CVD risk factors and adiposity and insulin resistance markers. This suggests that IGR (particularly DM) is related to IMT through mechanisms other than those conveyed by these factors.

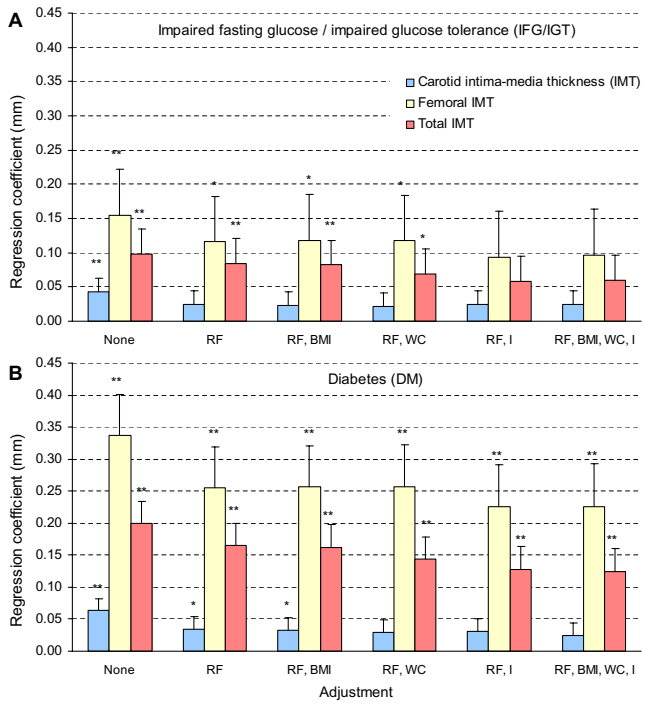

Figure 1

Associations between intima-media thickness (IMT) and categories of impaired glucose metabolism upon incremental adjustment for covariates (regression coefficients with their standard errors). Panel A: impaired fasting glucose/impaired glucose tolerance (IFG/IGT); Panel B: diabetes (DM). * p: 0.05–0.09. ** p < 0.05. All associations between impaired fasting glucose/normal glucose tolerance (IFG/NGT) and IMT were statistically not significant and are thus not shown. None: no adjustment; RF: major risk factors (low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, systolic blood pressure, smoking); BMI: body mass index; WC: waist circumference; I: serum fasting insulin.

Using HOMA instead of insulin for analyses shown in Table 2 and Figure 1 resulted in slightly different regression coefficients for HOMA than insulin in some instances although results were almost identical in many instances (results not shown).

Discussion

We found that pre-diabetes was associated with worsened levels of several major CVD risk factors as well as with increased carotid and femoral IMT independently of markers of adiposity (waist and BMI) or a marker of insulin resistance (insulin).

The smaller than expected effect of waist/BMI and insulin in explaining the relationships between IGR and the major CVD risk factors deserves several comments. First, we observed that BMI, waist or insulin attenuated the relationship between IGR categories to a fairly similar small extent and that adjusting for these factors altogether did not add substantial adjustment as compared to adjustment based on any of the three markers alone. This suggests that these three markers represent a same dimension/mechanism (e.g. insulin resistance) and that none of these markers conveys a substantial advantage in representing this dimension. In our results, waist tended however to perform slightly better than BMI. An advantage of waist over BMI has been found in some studies [33], but not all [34]. The fairly similar non-additive effect of the three considered markers (insulin, BMI, waist) has practical clinical relevance: BMI (or waist) is much simpler and less expensive to asses as compared to insulin and BMI (or waist) might be preferred to insulin for risk stratification, particularly in resource-constrained settings.

Several factors may underlie the smaller than expected effect of the considered markers (BMI, waist, insulin) on the relationship between IGR and major CVD risk factors. First, this may reflect the known fact that only a sub-group of obese persons are insulin resistant and are at risk for developing IGR [35] and that obese individuals without insulin resistance have only marginally increased CVD risk [35,36]. Second, BMI, waist and insulin may only imperfectly represent insulin resistance and the effect of such markers on the relationship between IGR and major CVD risk factors might have been larger, had we used better indicators of insulin resistance. However, better markers of insulin resistance, such as the euglycemic clamp, would require complex measurements that are not practical for epidemiological studies or usual clinical practice. Third, insulin resistance is a broad description underlying many different altered physiological factors. In particular, insulin resistance has been associated with a variety of pathophysiological effects related to abdominal fat, including cytokines secretion and inflammatory cell migration [37]. For instance, the proinflammatory cytokine TNF-alpha may impair intracellular insulin signaling independently of insulin [37]. BMI, waist and insulin may therefore only poorly correlate with such finer physiological factors. In particular, insulin may not be a reliable marker for insulin resistance and subsequent atherosclerosis [38]. This could particularly be the case in diabetic persons with depleted insulin secretion (whether type 1 or type 2 DM). However, a moderate effect of adiposity/insulin in explaining the relationship between IGR and major CVD risk factors is at odds with trials showing decreased incidence of DM among pre-diabetic persons who decreased their weight through lifestyle interventions [39] or through bariatric surgery [40]. A possible explanation underlying this contradiction is that these interventions not only decreased fat mass or insulin but also improved other factors which may directly improve insulin resistance, e.g. physical activity, nutritional patterns or secretion/action of several hormones (e.g. incretins).

Similarly to the association with major CVD risk factors, the relationship between IGR and IMT was only partially explained by waist, BMI and insulin. In other studies, carotid IMT was associated with 2hBG but with neither fasting glycemia nor a insulin sensitivity index [41,42]. Consistent with our results, IMT remained significantly associated with 2hBG upon adjustment for major CVD risk factors, waist, BMI and insulin sensitivity index [41]. Interestingly, we found that the relationships between IGR and IMT tended to be larger at femoral than carotid levels independently of adjustment. We are not aware of any other study that has investigated these associations between IGR and IMT at femoral level. However it was recently shown that metabolic syndrome components impacted selectively on IMT at the femoral site: insulin and triglyceride concentrations were strongly associated with femoral IMT but not with carotid IMT [18]. These findings suggest the usefulness of femoral IMT for assessing CVD outcomes [18,43].

In addition, the relationships between IGR and either major CVD risk factors or IMT may be linked to several specific mechanisms. It is shown that atherosclerosis is accelerated by insulin resistance and DM [38]. Atherosclerosis, which also encompasses an inflammatory process [44], is in turn related to several factors associated with adiposity and/or insulin resistance. These factors include numerous adipokines [45] that are released or modulated by adipose tissue. From another perspective, insulin resistance has also been shown to alter the endothelial function, which can result in impaired production of NO [46], reduced blood flow, pro-inflammatory state and prothrombotic state [46,47]. Furthermore, hyperglycemia can also induce atherosclerosis independently of insulin, e.g. through glycation of proteins and lipids and by increasing oxidative stress [44].

We found that IGT was associated with major CVD risk factors or IMT more strongly than IFG. This different significance of IFG and IGT is consistent with other studies showing a stronger association of IGT than IFG with CVD risk factors [48] and with IMT [41,49], but not with a recent prospective study linking CVD and total mortality at least as strongly with IFG as with IGT [7]. A greater impact of IGT than IFG may relate to the facts that subjects with IFG and IGT are more likely to be insulin resistant whereas subjects with IFG and NGT are more likely to have insufficient insulin secretion [48,50,51]. It has been

suggested that a pro-inflammatory state leading to CVD seems restricted to those individuals with IGR who are insulin resistant, measured by an insulin sensitivity index [52] or HOMA-IR [53].

Several limitations of this study need do be considered. First, we did not perform OGTT in individuals with FBG < 5.6 mmol/l and we could have missed a few cases of DM and IGT in persons with NFG but pathologically high 2hBG. The number of such cases is however expected to be small [54]. A second limitation is related to the cross-sectional nature of our study which precludes defining causal relationships. Third, we may not have captured insulin resistance optimally with BMI, waist and insulin. insulin was shown to relate only moderately with insulin resistance measured by euglycemic clamp [55]. Also, BMI and waist are only proxy measures of total adiposity and intra-abdominal adiposity. Yet, this study adds to the limited information on the associations between pre diabetes, on the one hand, and CVD risk factors and peripheral artery IMT, on the other hand. We are not aware of any previous study that has examined this issue in a population in the African region.

Conclusion

Our data show that IGT and to a lesser extent IFG (in addition to DM) are associated with impaired cardiovascular conditions (whether risk factors or IMT) and that these associations are only moderately mediated by markers of adiposity and insulin resistance. These findings provide further evidence for increased cardiovascular risk associated with pre-diabetes (the increased cardiovascular risk associated with DM is well established) and further stress the need for early screening and management of pre-diabetes.

Abbreviations

BMI: body mass index; BP: blood pressure; CRP: C-reactive protein. CVD: cardiovascular disease; DM: diabetes mellitus; FBG: fasting blood glucose; HDL-C: high-density lipoprotein cholesterol; HOMA-IR: homeostasis model assessment of insulin resistance; IFG: impaired fasting glucose; IGT: impaired glucose tolerance; IMT: intima-media thickness; LDL-C: low density lipoprotein cholesterol; OGTT: oral glucose tolerance test; 2hBG: 2-hour postload blood glucose

Competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

DF led the analysis of the data and the write up of the manuscript. JW coordinated several aspects of the survey and reviewed the manuscript. PY conducted ultrasonography on all participants and reviewed the manuscript. FP assisted in the interpretation of data and reviewed the manuscript. PB led the organization of the survey, assisted with the analysis and interpretation of the data and with the write up of the manuscript. All authors read and approved the final manuscript.

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Research article

Clustering of smoking, alcohol drinking and cannabis use in adolescents in a rapidly developing country

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Abstract

Background: Smoking, alcohol drinking and cannabis use ("risk behaviors") are often initiated at a young age but few epidemiological studies have assessed their joined prevalence in children in developing countries. This study aims at examining the joint prevalence of these behaviors in adolescents in the Seychelles, a rapidly developing country in the Indian Ocean.

Methods: Cross-sectional survey in a representative sample of secondary school students using an anonymous self-administered questionnaire (Global Youth Tobacco Survey). The questionnaire was completed by 1,321 (92%) of 1,442 eligible students aged 11 to 17 years. Main variables of interest included smoking cigarettes on ≥1 day in the past 30 days; drinking any alcohol beverage on ≥1 day in the past 30 days and using cannabis at least once in the past 12 months.

Results: In boys and girls, respectively, prevalence (95% CI) was 30% (26–34)/21% (18–25) for smoking, 49% (45–54)/48% (43–52) for drinking, and 17% (15–20)/8% (6–10) for cannabis use. The prevalence of all these behaviors increased with age. Smokers were two times more likely than non-smokers to drink and nine times more likely to use cannabis. Drinkers were three times more likely than non-drinkers to smoke or to use cannabis. Comparison of observed versus expected frequencies of combination categories demonstrated clustering of these risk behaviors in students ($P < 0.001$).

Conclusion: Smoking, drinking and cannabis use were common and clustered among adolescents of a rapidly developing country. These findings stress the need for early and integrated prevention programs.

Background

In addition to the increased risk of chronic diseases at an older age, smoking, drinking and use of illegal substances in adolescents are associated with more immediate health hazards such as depression, interpersonal violence, motor vehicle crashes and drowning, risky sexual behaviors, and suicidal behavior [1-3]. Furthermore, behaviors initiated during adolescence tend to track into adulthood [4]. Early

experience with smoking and drinking increases the risk of subsequent tobacco [5] and alcohol [6] dependences. In addition, cross-sectional [7-9] and longitudinal [10-12] studies in western countries have shown that these behaviors tended to cluster in adolescence and perhaps even at an earlier age [13]. Also of importance, these behaviors increase the likelihood to adopt other risk behaviors at a later age, such as multiple substance use, violence and delinquency [14,15].

The Republic of Seychelles comprises over 115 islands lying in the Indian Ocean, approximately 1,000 km east to Kenya and the country is part of the African region. Almost all of the population lives on three main islands and 90% reside on the largest one, Mahé. The large majority is of African descent. The country has experienced rapid socio-economic development due to booming tourism and fishing industries. The gross domestic product per capita has increased, in real terms, from US$ 2,927 in 1980 to US$ 5,239 in 2004.

Surveys among adults have shown high prevalence of both smoking and drinking in men but not in women [16-18]. In 1994, 51% of men and 6% of women reported drinking at least once per week [17] while 31% of men and 4% of women reported smoking daily in 2004 [18]. Until 2004, drugs such as heroin or cocaine were virtually unseen in the country, which was reflected by only very few police cases or hospital admissions related to illegal substance abuse.

In Seychelles, the law prohibits the sale of alcohol and cigarettes to children aged less than 18 years. Use and possession of cannabis and other illegal drugs is prohibited and liable to severe penalties. Both alcohol and cigarettes are heavily taxed and expensive. Advertising for cigarettes is banned while advertising for alcohol beverages is limited. Use of tobacco or alcohol in the premises of all schools is prohibited and severely sanctioned by school policies. Over the past decade, the ministry of health and some other organizations have been conducting high-profile awareness campaigns related to smoking, illegal drugs and responsible drinking. However, alcohol drinking is common at the occasion of social events and there has been a long standing social tolerance, at least among men [17].

The prevalence, age of onset and clustering of smoking, drinking and substance use in adolescents has been well described in western countries but few data on the joint prevalence of these behaviors are available from developing countries. In this study, we examined the prevalence and clustering patterns of smoking, drinking and cannabis use among a representative sample of school students aged 11–17 years of the Seychelles, a middle-income country experiencing rapid epidemiological transition.

Methods

This study is part of the Global Youth Tobacco Survey (GYTS), an international school-based survey of tobacco use that focuses on adolescents aged 13–15. The survey, which is sponsored by the World Health Organization (WHO) and the Centers for Disease Control (CDC), has been conducted once or several times in more than a hundred countries worldwide [19-22].

The GYTS is intended to be performed in students aged 13–15 years. In Seychelles this includes the four secondary grades S1–S4 (school is compulsory through the S4 level). There are 12 secondary schools (10 public, 2 private) on the three main islands that teach grades S1–S4. The total enrolment of Grades S1–S4 for these 12 schools was 6,161. A two-stage cluster sample design was used to produce a representative sample of all students in grades S1–S4 from all public and private schools in Seychelles. The first-stage sampling frame consisted of all schools containing the grades S1, S2, S3, and S4. Schools were selected with probability proportional to school enrolment size. The second-stage sampling frame consisted of an equal-probability sampling (with a random start) of all S1–S4 classes from the selected schools. Sample size estimation showed that 1,224 completed interviews were needed from an enrolment of 6,161 for a ± 5% margin of error.

Eight percent of students did not participate in the study, because they were absent on the day the study took place for benign reasons (e.g. illness) or because they were suspended for disciplinary reasons (i.e. major misbehaviors) [23].

The questionnaire was anonymous, self-administered, and written in English (English is the main language used at school). The questionnaire included 56 core questions on tobacco and other variables (for example age, sex) used in all GYTS worldwide. Fifteen additional questions were included in order to assess alcohol drinking and the use of illegal drugs [see Additional file 1]. Most students could complete the questionnaire within 35–45 minutes. The survey took place at the same time in all selected classes. The students and parents were not informed prior to the survey in view of the non-sensitive nature of the survey, the absence of invasive investigations or physical measurements, the allowance for declining participation given to all children, and the anonymous nature of the questionnaire ensuring confidentiality of all answers by all students. The research committee of the Ministry of Health and the Ministry of Education approved the study includ-

Table 1: Prevalence (percent with their 95% confidence intervals) of students reporting risk behaviors, by sex and age

	Age	11–13 years		14 years		15–17 years		Total	
		%	95% CI	%	95% CI	%	95% CI	%	95% CI
Boys	n	257		160		203		620	
	Ever smoking	46	38 - 54	53	45 - 61	67	60 - 73	54	50 - 59
	Smoking in the past month	27	20 - 35	24	18 - 31	38	30 - 45	30	26 - 34
	Drinking in the past month	45	38 - 59	40	35 - 46	63	56 - 69	49	45 - 54
	Ever drunkenness	40	33 - 47	44	35 - 53	58	50 - 66	47	42 - 61
	Ever cannabis	15	10 - 22	15	11 - 21	29	23 - 37	20	17 - 22
	Cannabis in the past year	15	10 - 19	12	8 - 18	26	20 - 33	17	15 - 20
Girls	n	274		193		187		654	
	Ever smoking	30	24 - 36	44	36 - 52	56	50 - 62	41	38 - 45
	Smoking in the past month	15	10 - 22	23	18 - 29	28	22 - 35	21	18 - 25
	Drinking in the past month	41	34 - 48	47	39 - 55	58	49 - 65	48	43 - 52
	Ever drunkenness	35	29 - 42	44	37 - 51	52	45 - 60	43	39 - 47
	Ever cannabis	6	4 - 9	6	3 - 12	16	12 - 21	9	7 - 11
	Cannabis in the past year	6	4 - 10	5	2 - 11	12	9 - 18	8	6 - 10

ing the questionnaire and the fact that informed consent by parents was not necessary.

Some questions of particular relevance for this study read as follows: "During the past 30 days, on how many days did you smoke cigarettes?". Consistent with the GYTS methodology, "smoking in the past month" was defined as smoking on one day per month or more. "Have you ever tried cigarette smoking, even one or two puffs?". "Ever smoking" was defined as having ever smoked a cigarette, even a puff, at least once. "During the past 30 days, on how many days did you drink alcohol?". "Drinking in the past month" was defined as any alcohol consumption on at least one day during the past 30 days. "Have you ever had so much alcohol that you were drunk?". "Ever drunkenness" was defined as having ever been drunk at least once. "Did you ever take a joint, marijuana, or hashish in your life?". "Ever cannabis" was defined as having ever used cannabis at least once. "During the past 12 months, how many times did you take a joint, marijuana, or hashish?". "Cannabis in the past year" was defined as having smoked cannabis at least once during the past 12 months [see Additional file 1].

Overall prevalence estimates and 95% confidence intervals were weighted consistent with the survey design. We selected age categories (11–13, 14 and 15–17) so that numbers of students were balanced across all categories in order to maximize the power of statistical analyses. Differences in prevalence estimates were tested using the chi-square test. To analyze clustering patterns, we calculated the expected frequencies of the 4 different possible combinations of the three risk factors (i.e. 0, 1, 2, or 3) given the observed prevalence of each risk factor and assuming independency in associations. Difference between observed versus expected prevalences of combinations was tested with a goodness-of-fit chi square with 3 degrees of freedom. Analyses were conducted with Stata 8.2. P-values less than 0.05 were considered significant.

Results

All schools agreed to participate (100% school response rate) and 1,321 (92%) of 1,442 eligible students aged 11 to 17 years completed the questionnaire. Forty-two percent of all participants were aged 11–13, 28% aged 14, and 30% aged 15–17. The proportions of respondents did not differ significantly by age and age category. The answers not completed in the questionnaire were coded as missing values and their proportions varied between 0.9% and 5.6% depending on the questions.

Table 1 presents the proportions of boys and girls reporting risk behaviors. The prevalence of risk behaviors was markedly higher in the older than younger age categories. This suggests an important uptake of these risk behaviors over the considered age categories. The prevalence of risk behaviors was higher in boys than girls. However, at age 15–17, the difference between boys and girls was only minimal for monthly smoking and for monthly drinking, suggesting convergence in the prevalence in late adolescence. The prevalence of cannabis use in the past year was larger in boys than in girls in all age categories.

Table 2 shows the proportions of combinations of risk behaviors by sex and age categories. The proportions of students reporting none of the three risk behaviors tended to be lower in boys than in girls and in younger than older students (e.g. 41% at age 11–13 and 24% at age 15–17 among boys, respectively 50% and 35% among girls of same age categories). Inversely, the proportions of students reporting the three risk behaviors tended to be higher in boys than in girls and in the older than younger

Table 2: Prevalence (percent with their 95% confidence intervals) of students reporting various combinations of smoking in the past month (S), drinking in the past month (D) and cannabis use in the past year (C) by sex and age. Y: Yes, N: No

	Age				11–13 years		14 years		15–17 years	
	S	D	C	n	%	95% CI	%	95% CI	%	95% CI
Boys	N	N	N	226	41	34–49	44	36–52	24	18–30
	Y	N	N	31	5	3–9	5	2–10	5	3–10
	N	Y	N	135	20	15–27	18	13–24	27	20–35
	N	N	Y	14	2	1–4	4	2–9	2	1–6
	Y	Y	N	58	9	6–13	10	7–15	9	6–14
	N	Y	Y	12	2	1–4	1	0–5	3	1–7
	Y	N	Y	11	1	0–3	1	0–5	4	2–7
	Y	Y	Y	54	7	4–11	5	2–11	14	10–20
Girls	N	N	N	286	50	43–57	43	36–50	35	27–43
	Y	N	N	20	3	1–7	3	1–7	4	2–8
	N	Y	N	180	25	20–32	28	21–35	31	25–38
	N	N	Y	19	4	2–8	4	2–7	1	0–4
	Y	Y	N	72	7	4–13	14	10–20	13	9–18
	N	Y	Y	5	1	0–3	1	0–4	1	0–4
	Y	N	Y	4	<1	0–3	1	0–4	1	0–4
	Y	Y	Y	28	3	1–6	3	1–8	8	5–13

age categories (e.g. 7% at age 11–13 and 14% at age 15–17 among boys, respectively 3% and 8% among girls in the same age categories).

The figure shows the prevalence of combinations of 0, 1, 2 and 3 risk behaviors among all students. The prevalence of expected and observed combinations categories differed significantly ($P < 0.001$), which demonstrates a clustering pattern of the considered risk behaviors. Observed prevalences were higher than expected prevalences in the extreme combination categories (respectively 0 and 3 risk behaviors) while the reverse was found in the intermediate combination categories (respectively 1 and 2 risk behaviors). Compared to the expected prevalence, the observed prevalence was +33% (45% vs. 34%), -30% (34% vs. 48%), -15% (14% vs. 17%) and +380% (7% vs. 2%) in the combination categories of 0, 1, 2 and 3 risk behaviors, respectively. Results did not differ significantly by sex or age (data not shown).

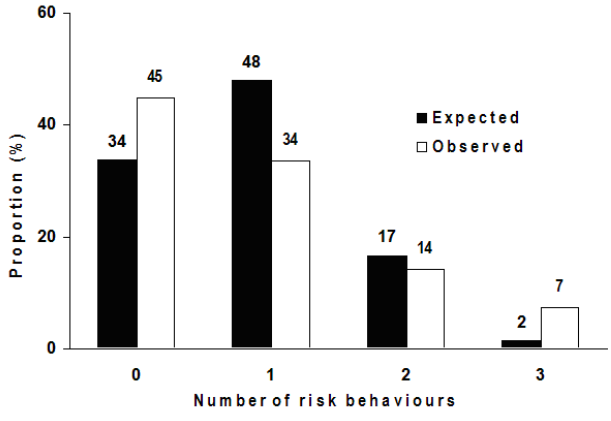

Figure 1. Prevalence of expected and observed combinations of risk behaviors (smoking, drinking and cannabis use) in students aged 11–17

Table 3 shows the joint prevalence of risk behaviors among smokers (n = 294), drinkers (n = 613) and cannabis users (n = 154). Compared to non-smokers, smokers were two times more likely to drink (76% vs. 38%), and more than nine times more likely to use cannabis (35% vs. 4%). Compared to non-drinkers, drinkers were three times more likely to smoke (39% vs. 11%) or to use cannabis (20% vs. 6%). Compared to non-users, cannabis users were four time more likely to smoke (75% vs. 18%) and two times more likely to drink (77% vs. 44%). Among drinkers, there were twice as many smokers as cannabis users (39% vs. 20%) and cannabis use was more common among smokers than among drinkers (35% vs. 20%). The 95% confidence intervals of prevalence estimates by "yes" and "no" status fell largely apart of each other in all situations shown in the table, which translates into significant differences in all instances.

Table 4 presents selected recently published estimates of the gender-specific prevalence of smoking, drinking and cannabis use among youth the United States [24], England [25], Switzerland [26], and South Africa [20,27], in addition to our findings in Seychelles. We did not find publications on the joint prevalence of the considered behaviors in representative samples in African countries except for one in South Africa. Although the mean age of the study populations and the criteria used to define risk behaviors differ across countries, – and the figures must therefore be interpreted with great caution -, the table may be useful to set the prevalence of risk behaviors among Seychelles youth in an international context. The data suggest that the prevalence and gender patterns for smoking and drinking are not largely different in Seychelles as compared to the considered western countries. However fewer youths seemed to use cannabis in the former than in the latter.

Discussion

We found high prevalences of smoking, drinking and cannabis use among adolescents in the Seychelles. These risk behaviors tended to cluster, particularly smoking and drinking and smoking and cannabis.

Comparisons between countries must be interpreted cautiously in view of different methodology used across countries. However, the prevalence of smoking seemed

Table 3: Joint prevalence of risk behaviors by sex

		Smokers			Drinkers			Cannabis users		
	n	Yes (%)	No (%)	Ratio	Yes (%)	No (%)	Ratio	Yes (%)	No (%)	Ratio
Smoking in the past month										
Boys	164	100	-	-	43	16	2.7	76	20	3.8
Girls	130	100	-	-	35	8	4.7	75	17	4.5
Total	294	100	-	-	39	11	3.4	75	18	4.1
Drinking in the past month										
Boys	304	71	39	1.8	100	-	-	76	44	1.7
Girls	309	81	39	2.1	100	-	-	77	45	1.7
Total	613	76	38	2.0	100	-	-	77	44	1.7
Cannabis in the past year										
Boys	105	42	6	7.6	26	8	3.2	100	-	-
Girls	49	26	2	11.4	12	3	3.7	100	-	-
Total	154	35	4	9.3	20	6	3.5	100	-	-

Prevalence of risk behaviors by "Yes" vs. "No" status differed significantly ($p < 0.05$) in all instances. Smokers: smoking in the past month; Drinkers: drinking in the past month; Cannabis users: Cannabis use in the past year.

higher in Seychelles than in several low-income developing countries in the African region [20], but similar as compared to western countries such as Switzerland [28], the US [24] or the UK [25]. Higher prevalence in Seychelles as compared to Africa may reflect a larger purchasing power of youth in Seychelles than in many other African countries [29]. While smoking is much less common in female than in male adults in Seychelles (respectively 4% and 31% in 2004 [30]), the much smaller difference by gender among youth may predict a marked increase in the prevalence of smoking in future generations of adult women [17,31]. A lack of gender differences in adolescent smoking has been consistently found in developed and developing countries (Table 4) [24,25,28,32].

Drinking in adolescents was as frequent in Seychelles as in South Africa [27] or in selected western countries [24,25,28]. The lack of a gender difference in drinking prevalence in Seychellois adolescents, consistent with findings in industrialized countries (Table 4) [24,25,28,32], may announce a convergence by gender among younger cohorts, in contrast to the currently much higher prevalence of drinking in male than female adults [16-18].

Fewer adolescents in Seychelles reported cannabis use during the past year compared to youths in South Africa or in some western countries (Table 4). A higher prevalence in boys than in girls in Seychelles contrasts with the lack of a gender difference observed in selected other countries. The situation may be consistent with the indirect evidence of a low use of other illegal drugs in Seychelles at the time of the survey.

We found that smoking, drinking and cannabis use tended to cluster. The association was particularly strong between smoking and cannabis use, which is consistent with a clustering pattern often found in developed countries [1,33]. The cross-sectional nature of our data precludes concluding on the direction of these associations (whether a certain behavior precedes or follows another behavior). Studies investigating temporal relationships of substance use suggest that smoking often precedes alcohol drinking and cannabis use and that smoking may represent a "gateway" to other substance use [14,15,34-37]. On the other hand, a "problem behaviors theory" postulates that there is no clear temporal sequence in the use of different substances. Along this theory, adoption of risk behaviors such as smoking, drinking, cannabis use and the use of other drugs is the consequence of a single underlying characteristic [38], e.g. an affinity to sensation-seeking and risky behaviors [39]. The associations between risk behaviors may also reflect social circumstances such as use of substances by peers and a perception by children that use of substances is encouraged among adults [40]. Pharmacologic factors may also play a role: tobacco, alcohol, cannabis and other drugs impact on several common neurotransmitters [41]. Moreover, early onset of smoking, drinking or cannabis use has been associated with higher nicotine addiction [4], higher alcohol dependence [6] and increased problem behaviors [42] during later adolescence, which may underlie other mechanisms for the association of these behaviors.

Mirroring a higher than expected prevalence of adolescents with all three behaviors, we also found a larger than expected proportion of adolescents indulging in none of the three risk behaviors. One could speculate that abstain-

Table 4: Prevalence of risk behaviors in adolescents in Seychelles and in selected countries

	Proportion (%)			
	Age (y)	Boys	Girls	Total
Ever smoking				
Seychelles*	11–17	54	41	48
South Africa (a)	13–15	-	-	44
England£	11–15	37	41	39
USA$	14–18	59	58	58
Switzerland&	15	64	64	64
At least 1 cigarette in the past month				
Seychelles	11–17	30	21	25
South Africa (a)	13–15	-	-	18
USA	14–18	30	35	28
England[1]	11–15	11	17	14
Switzerland	15	22	21	21
At least 1 drink in the past month				
Seychelles	11–17	49	48	48
South Africa (b)	16–17	50	32	-
USA	14–18	44	46	45
England	11–15	37	39	38
Switzerland	15	77	74	75
Ever drunkenness				
Seychelles	11–17	47	43	45
USA[2]	14–18	29	28	28
England[3]	11–15	42	50	46
Switzerland	15	64	53	58
Ever used cannabis				
Seychelles	11–17	20	9	14
South Africa (b)	16–17	32	13	-
USA	14–18	43	38	40
Switzerland	15	44	36	40
Cannabis use in the past year				
Seychelles	11–17	17	8	12
South Africa (b)	16–17	22	9	-
USA	14–18	25	19	22
England	11–15	12	10	11
Switzerland	15	35	28	31

*Seychelles: n = 1,321, 2002.
£England: n = 9,572, 2004, reference [25]; $USA: n = 15,214, 2003, reference [24].
&Switzerland: n = 2,613, reference [26]
South Africa (a): n = 2,579, 2002, reference [20]; South Africa (b): Cape Town, n = 2,732, 1997–2001, reference [27].
[1] Regular occasional smoking.
[2] At least 5 drinks in a row.
[3] Among students who drank in the past week.

ers might include adolescents who socialize less and are therefore less exposed to peer pressure from experimenters or who are submitted to more parental or other social or cultural forms of control. Alternatively, abstainers might have developed particularly strong resilience skills, in relation to personal characteristics or to an enabling familial or social milieu.

Our study has some limitations. The sample size of the survey limits the precision of the prevalence estimates in some categories with subsequent large confidence intervals (e.g. cannabis use among girls). The size of the samples if further limited by missing data in some students (typically up to 5%), a factor that cannot be avoided from self-reported questionnaires. There are also several sources of bias in prevalence estimates. First, students could exaggerate or underestimate their answers in a systematic manner. Although the extent of this potential bias cannot be determined, questions on tobacco were shown to have good test-retest reliability in a another study [20]. Second, the prevalence of risk behaviors may be underestimated since non-participants typically indulge in more detrimental behaviors as compared to participants [43], as we have also shown on this sample of students [23]. On

the other hand, strong points of the study include the large participation, the population-based design, and the anonymous nature of the data collected.

Our findings have several public health implications. First, the early onset of the considered behaviors emphasizes the importance of implementing school-based surveillance systems for guiding and evaluating policy and prevention programs.

Second, given the much lower prevalence of smoking and drinking in women compared to men in Seychelles (as found in many other developing countries), the only small gender difference found in boys and girls may predict a convergence by sex of these behaviors over the next decades. However, further appropriately powered studies (i.e. studies with larger sample sizes and extended age categories) should examine trends and significance of such gender patterns over age in adolescence before conclusions can be drawn. These issues are important as they underlie specific target groups for prevention.

Third, given tracking into adulthood [4,6], the fairly high prevalence of the considered risk behaviors in adolescents emphasizes the need to strengthen school-based programs and policies aimed at promoting healthy behaviors as a main strategy to tackle non-communicable diseases in the general population. It has been shown that delaying smoking onset until after the age of 13 can reduce the prevalence of adult smoking [44] and that delaying drinking onset after the age of 14 can reduce the risk of alcohol dependence [6]. More generally, it is believed that adoption of healthy behaviors early in life has the potential to reduce CVD in adulthood [45]. Furthermore, programs to prevent substance use should therefore not be restricted to adolescents only but also extend to pupils in elementary school.

Fourth, the trend towards clustering of smoking, drinking and use of cannabis, as found in this report and in other studies, emphasizes the need to address these behaviors within comprehensive and integrated programs [44]. Similarly, comprehensive benefits can be expected from various policy measures: high cigarette prices can reduce the prevalence of both drinking and cannabis use in adolescents [46].

Conclusion

We found that smoking, drinking and cannabis use are common among adolescents of a rapidly developing country and that these risk behaviors are adopted at an early age and tend to cluster. These findings stress the need to initiate prevention interventions at an early age and using integrated approaches.

Competing interests

The author(s) declare that they have no competing interests.

Abbreviations

GYTS: Global Youth Tobacco Survey

WHO: World Health Organization

CDC: Centers for Disease Control

Authors' contributions

DF carried out literature research, statistical analysis, assisted in interpretation of the data, and was the main writer of the manuscript. BV participated in the study design and collected the data. AC and WW assisted in statistical analysis and reviewed the manuscript. PB designed the study, carried out analysis and interpretation of the data, and wrote partially the report.

Additional material

Additional file 1

Final-GYTS-questionnaire(17sep02). Global Youth Tobacco Survey (GYTS) questionnaire with additional questions on alcohol consumption and use of illegal substances.
Click here for file
[http://www.biomedcentral.com/content/supplementary/1471-2458-6-169-S1.pdf]

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Effect of Fructose Overfeeding and Fish Oil Administration on Hepatic De Novo Lipogenesis and Insulin Sensitivity in Healthy Men

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High-fructose diet stimulates hepatic de novo lipogenesis (DNL) and causes hypertriglyceridemia and insulin resistance in rodents. Fructose-induced insulin resistance may be secondary to alterations of lipid metabolism. In contrast, fish oil supplementation decreases triglycerides and may improve insulin resistance. Therefore, we studied the effect of high-fructose diet and fish oil on DNL and VLDL triglycerides and their impact on insulin resistance. Seven normal men were studied on four occasions: after fish oil (7.2 g/day) for 28 days; a 6-day high-fructose diet (corresponding to an extra 25% of total calories); fish oil plus high-fructose diet; and control conditions. Following each condition, fasting fractional DNL and endogenous glucose production (EGP) were evaluated using [1-^{13}C]sodium acetate and 6,6-$^{2}H_2$ glucose and a two-step hyperinsulinemic-euglycemic clamp was performed to assess insulin sensitivity. High-fructose diet significantly increased fasting glycemia (7 ± 2%), triglycerides (79 ± 22%), fractional DNL (sixfold), and EGP (14 ± 3%, all $P < 0.05$). It also impaired insulin-induced suppression of adipose tissue lipolysis and EGP ($P < 0.05$) but had no effect on whole-body insulin-mediated glucose disposal. Fish oil significantly decreased triglycerides (37%, $P < 0.05$) after high-fructose diet compared with high-fructose diet without fish oil and tended to reduce DNL but had no other significant effect. In conclusion, high-fructose diet induced dyslipidemia and hepatic and adipose tissue insulin resistance. Fish oil reversed dyslipidemia but not insulin resistance. *Diabetes* 54:1907–1913, 2005

Over the past decades, per capita consumption of high-fructose corn syrup has increased dramatically. Several authors suggest that increased fructose ingestion may be responsible for the present epidemic of obesity and the increased incidence of metabolic syndrome and diabetes (1). Diets rich in simple sugars, particularly fructose, have been shown to be associated with hypertriglyceridemia both in humans (2) and rodents (3). This may be due to stimulation of hepatic de novo lipogenesis (DNL) and increased secretion of triglyceride-rich particles by the liver or to decreased extrahepatic clearance of triglyceride particles (4,5). Moreover, there is evidence that high-fructose diets can lead to insulin resistance in rodents (3,6). To further delineate the metabolic consequences of fructose overconsumption, we measured fractional hepatic DNL and insulin sensitivity in the liver, adipose tissue, and at the whole-body level. This was performed in a group of healthy male volunteers after 6 days of either fructose overfeeding or an isoenergetic, low-fructose diet. Since n-3 polyunsaturated fatty acids are known to prevent hypertriglyceridemia and the development of insulin resistance in dietary models of obesity in rats (6) and may suppress hepatic lipogenic enzymes (7), each participant was also studied after a 4-week fish oil supplement.

RESEARCH DESIGN AND METHODS

Seven healthy male volunteers without family history of diabetes were recruited by advertisement. They were aged 22–31 years and had BMIs of 20.2–25.4 kg/m^2 (Table 1). All subjects were in apparent good health, were nonsmokers, and took no medications. The study was approved by the ethical committee of Lausanne University School of Medicine and a written consent was obtained from each subject after the nature of the study was explained.

Anthropometry and body composition measurements. Standing height was measured using a stadiometer. Body weight and hip and waist circumferences were measured before the last meal preceding each study (8). Body composition was estimated from subcutaneous skin fold thickness measurements at the biceps, triceps, subscapular, and suprailiac sites as described by Durnin and Womersley (9).

Study design. Each subject was studied on four occasions (Fig. 1). On one occasion, volunteers received 7.2 g of fish oil (1.2 g eicosapentaenoic acid and 0.8 g docasahexaenoic acid; Biorganic Omega-3, Gisand, Bern, Switzerland) per day for 28 days. It has been documented that such supplementation with fish oils leads to marked increases in n-3 fatty acids in serum phospholipids (10,11). On another occasion, subjects ingested 3 g of fructose (D-Fructose; Fluka Chemie Gmbh, Buchs, Switzerland) per kilogram of body weight per day (high-fructose diet) as a 20% fructose solution with the three main meals during the 6 days before the test. On the third occasion, fish oil supplementation was combined with high-fructose diet. Each subject also underwent a

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ChREBP, carbohydrate response element–binding protein; DNL, de novo lipogenesis; EGP, endogenous glucose production; NEFA, nonesterified fatty acid.

TABLE 1
Subject characteristics

	Controls	Fish oil	High-fructose diet	High-fructose diet plus fish oil
Protein/fat/carbohydrate (%)*	15/35/50	15/35/50	11/26/63	11/26/63
Body weight (kg)	71.5 ± 4.0	72.6 ± 3.7	72.1 ± 4.1	73.1 ± 4.1
Body fat (%)	16.5 ± 0.7	17.2 ± 0.7	16.5 ± 0.8	17.2 ± 1.0
Waist circumference (cm)	80.0 ± 2.9	81.1 ± 3.3	81.0 ± 2.7	81.2 ± 2.6
Fasting NEFA (μmol/l)	392 ± 43	375 ± 48	243 ± 43†	212 ± 26†
Percent of controls	100 ± 0	101 ± 14	61 ± 6†	55 ± 5†
Fasting insulin (pmol/l)	53 ± 7	49 ± 6	61 ± 9	58 ± 4
Percent of controls	100 ± 0	96 ± 11	117 ± 14	116 ± 13
Fasting glucose (mmol/l)	4.6 ± 0.1	4.7 ± 0.1	5.0 ± 0.1†	5.0 ± 0.1†
Percent of controls	100 ± 0	101 ± 4	107 ± 3†	108 ± 3†
Fasting lactate (mmol/l)	0.7 ± 0.1	0.7 ± 0.1	1.1 ± 0.1†	1.0 ± 0.1†
Percent of controls	100 ± 0	107 ± 9	158 ± 12†	141 ± 9†

Data are means ± SE. *Percentage of total energy from protein, fat, and carbohydrate. †Significantly different ($P < 0.05$).

control test. Volunteers were instructed to avoid certain foods and to follow a balanced, isoenergetic diet, which was controlled by a food diary during an initial 3-day period. Thereafter, during the 3 days preceding each test, the subject followed a provided isoenergetic diet (15% proteins, 35% lipids, 40% starch, 10% mono- and disaccharides) partitioned into three meals at 0700, 1200, and 1900 and two snacks at 0900 and 1500. During the high-fructose diet, subjects received the same diet supplemented with 3 g · kg^{-1} · day^{-1} fructose, resulting in an hyperenergetic (800–1,000 kcal/day) diet containing 11% proteins, 26% lipids, 30% starch, 8% glucose and disaccharides, and 25% fructose. Subjects were told to avoid vigorous physical activity during the 6 days preceding the tests.

The order by which the four dietary conditions were applied was randomized, with an interval of 12 weeks after the two tests with fish oil administration to allow wash-out of fish oil between the experiments.

Fasting hepatic DNL and endogenous glucose production. After each dietary condition, subjects underwent an overnight 13-h study (from 2200 to 1100 of the next morning), during which they stayed in bed and slept between 2200 and 0600. On the evening of the study, they took their last meal at 1830. At 2030, two indwelling catheters were inserted: one into a right wrist vein for blood sampling; the other into a vein of the controlateral forearm for infusions. From 2200 to 0730, 0.5 g/h of [1-^{13}C]sodium acetate (10 mg/ml in NaCl 0.9%) was infused constantly. Whole-body glucose turnover was assessed with 6,6-$^{2}H_2$ glucose infusion (bolus: 2 mg/kg; continuous: 20 μg · kg^{-1} · min^{-1}) between 0500 and 1100. Basal blood samples were obtained at 0600, 0700, and 0730 for determination of hepatic DNL, endogenous glucose production (EGP), insulin, glucose, nonesterified fatty acid (NEFA), and triglyceride concentrations (referred as "fasting condition").

Hyperinsulinemic-euglycemic clamp. To assess insulin sensitivity, insulin-mediated glucose disposal (6,6-$^{2}H_2$ glucose, "hot infusion model") (12), inhibition of EGP, and suppression of lipolysis (plasma NEFA concentrations) and lipid oxidation (indirect calorimetry), a two-step (0.2 mU · kg^{-1} · min^{-1} from $t = 0$ to $t = 90$, then 0.5 mU · kg^{-1} · min^{-1} from $t = 90$ to $t = 180$) hyperinsulinemic-euglycemic clamp (13) was performed between 0800 and 1100 (Fig. 1). Blood samples were collected at 30-min intervals to measure insulin, NEFA, and triglycerides concentrations. The glucose infusion rate (insulin resistance) during the clamp was used to evaluate insulin-mediated glucose disposal. Indirect calorimetry was performed using a ventilated canopy as described previously (14) from 0700 until 1100 (Fig. 1). Energy expenditure and substrate oxidation rates were measured using the equation of Livesey and Elia (15).

Analytical procedures. Blood samples were immediately centrifuged at 4°C to separate plasma, which was then frozen at −20°C until testing. Plasma glucose and lactate concentrations were measured with a glucose-lactate analyzer YSI 2300 STAT Plus (Yellow Springs, OH). Plasma NEFA and triglycerides concentrations were analyzed by a colorimetric method using commercial kits for NEFA (NEFA C; Wako Chemicals, Freiburg, Germany) and for triglycerides (Biomérieux Vitek, Switzerland). Commercial radioimmunoassay and enzyme-linked immunosorbent assay kits were used for determination of plasma insulin (Biochem Immunosystems, Freiburg, Germany), adiponectin (Linco Research, St. Charles, MO), and resistin (Human Resistin ELISA, BioVendor Laboratory Medicine, Czech Republic). During the clamp, plasma glucose concentrations were measured by the glucose oxidase method using a Beckman glucose analyzer II (Beckman Instruments, Fullerton, CA). Plasma 6,6- $^{2}H_2$ glucose isotopic enrichment was measured by gas chromatography–mass spectrometry (Model 5973; Hewlett-Packard, Palo Alto, CA) as described (16). Plasma VLDL-^{13}C palmitate enrichment and mass isotopomers were measured as described by Hellerstein et al. (17). Gas chromatography–mass spectrometry was used for analysis of isotopic enrichments of plasma fatty acid-methyl esters from VLDL. For fatty acid-methyl esters analysis, a 25-m fused DB-1 silica column was used, with electron impact ionization ion at m/z 270–272 representing the parent M0 through the M2 isotopomers (17). Fractional DNL was calculated by the isotopomer distribution analysis technique. The ratio of excess double-labeled to excess of single-labeled species (EM2/EM1) in VLDL palmitate reveals the isotope enrichment of the true precursor pool for lipogenesis (hepatic cytosolic acetyl-CoA) by application of probability logic based on the multinomial expansion. The fractional contribution from DNL to VLDL fatty acid can then be calculated by the precursor-product relationship (17).

Statistical analysis. Data are expressed as means ± SE. Statistical analyses were performed by using STATA version 8.2 (StataCorp, College Station, TX)

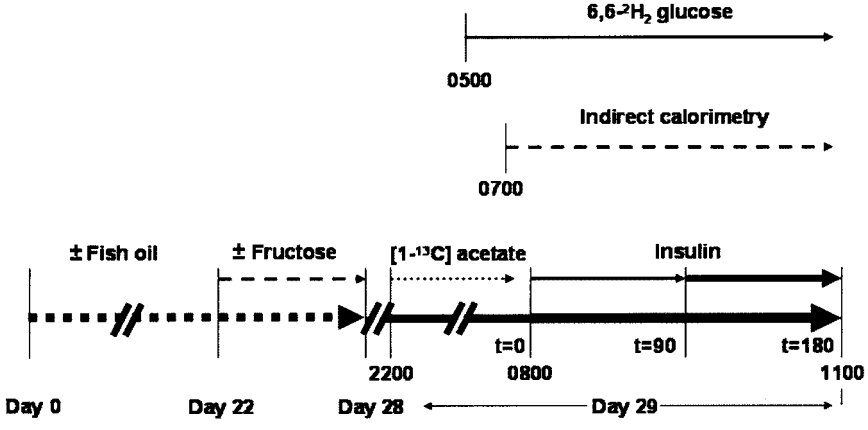

FIG. 1. Experimental protocol. After each of the four types of dietary intervention, a 13-h metabolic study was started at 2200. At 2200, 0.5 g/h of [1-^{13}C]acetate was perfused until 0730. 6,6-$^{2}H_2$ glucose (bolus: 2 mg/kg; continuous: 20 μg · kg^{-1} · min^{-1}) was perfused between 0500 and 1100. Indirect calorimetry was carried out from 0700 to 1100. Between 0800 ($t = 0$ min) and 1100 ($t = 180$ min), a two-step (0.2 mU · kg^{-1} · min^{-1} and 0.5 mU · kg^{-1} · min^{-1}) hyperinsulinemic-euglycemic (4.9 ± 0.1 mmol/l) clamp was performed.

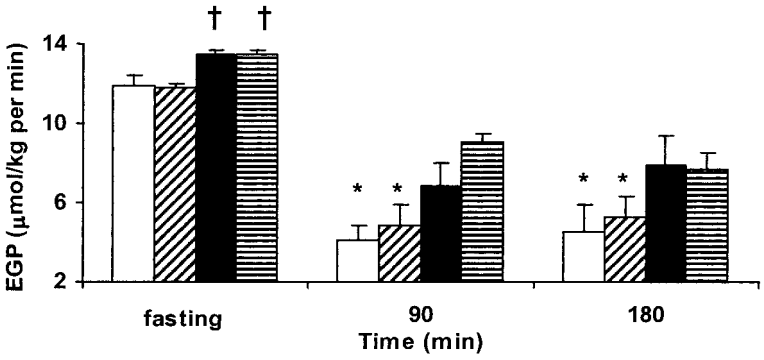

FIG. 2. EGP in fasting conditions and at 90 and 180 min of euglycemic-hyperinsulinemic clamping after control condition (□), after 28 days of fish oil supplementation (▨), after 6 days of high-fructose diet (■), and after 28 days of fish oil supplementation plus high-fructose diet (▤) in seven men. Values are means ± SE represented by vertical bars. *Significant suppression ($P < 0.05$) vs. fasting control subjects and fish oil alone. †$P < 0.05$ vs. fasting control subjects and fish oil alone.

with $P < 0.05$ as level of significance. Global mean difference among the various conditions was tested using the Friedman's test. When a significant difference was found, multiple comparisons between two conditions were performed using the paired Wilcoxon test. To test the existence of a trend, we performed the Page's test (18).

RESULTS

Baseline. Mean fasting parameters are summarized in Table 1. The subjects' body weights and compositions were not affected significantly by high-fructose diet or fish oil supplementation, respectively. Insulin levels did not differ significantly between the four conditions. Fasting glycemia and lactatemia significantly increased after high-fructose diet (7 ± 2 and 58 ± 12%, respectively; $P < 0.05$) compared with control condition, whereas fish oil had no influence.

As shown in Fig. 2, fasting EGP was significantly higher (14 ± 3%, $P < 0.05$) after the high-fructose diet (13.5 ± 0.2 μmol · kg^{-1} · min^{-1}) and the high-fructose diet plus fish oil (13.5 ± 1.4 μmol · kg^{-1} · min^{-1}) compared with fish oil alone (11.8 ± 1.3 μmol · kg^{-1} · min^{-1}) and control subjects (11.9 ± 0.5 μmol · kg^{-1} · min^{-1}). Fish oil did not change EGP significantly. Fasting NEFAs (Table 1) were significantly lower (−39 ± 6%, $P < 0.05$) after high-fructose diet (243 ± 43 μmol/l) compared with control subjects (392 ± 43 μmol/l). Again, fish oil had no significant impact on fasting NEFA concentrations. Fasting adiponectin and resistin concentrations were similar under all four conditions (adiponectin: control subjects, 7.7 ± 1.3; fish oil alone, 7.3 ± 1; high-fructose diet, 7.9 ± 1.4; high-fructose diet plus fish oil, 7.6 ± 1.5 μg/ml; resistin: control subjects, 2.2 ± 0.7; fish oil alone, 2.1 ± 0.4; high-fructose diet, 2.0 ± 0.5; high-fructose diet plus fish oil, 2.4 ± 0.5 ng/ml). Fasting glucose oxidation was higher (13.7 ± 1.8 and 13.8 ± 1.4 vs. 9.2 ± 2.2 and 7.7 ± 0.9 μmol · kg^{-1} · min^{-1}) and lipid oxidation lower (0.24 ± 0.12 and 0.23 ± 0.09 vs. 0.36 ± 0.12 and 0.61 ± 0.07 mg · kg^{-1} · min^{-1}) after high-fructose diet and high-fructose diet plus fish oil than after fish oil alone or control subjects ($P < 0.05$ for both). High-fructose diet increased fasting triglycerides (1.13 ± 0.21 mmol/l) by 79 ± 22% ($P < 0.05$) compared with control subjects (0.63 ± 0.07 mmol/l), as shown in Fig. 3. Fasting triglycerides concentrations were significantly lower (37%, $P < 0.05$) in the high-fructose diet plus fish oil (0.83 ± 0.12 mmol/l) compared with the high-fructose diet alone, but both remained significantly higher than fish oil alone, and control subjects ($P < 0.05$). Figure 4 shows the percentage of fractional DNL. After fish oil alone and control subjects, DNL was very low (1.9 ± 0.44% and 1.6 ± 0.34%, respectively). In contrast, DNL significantly increased after the high-fructose diet to 9.4 ± 2.8% ($P < 0.05$). Mean DNL tended to be lower after the high-fructose diet plus fish oil (mean DNL: 7.5 ± 1.8%) compared with the high-fructose diet alone but the difference was not significant. The relationship between DNL and fasting

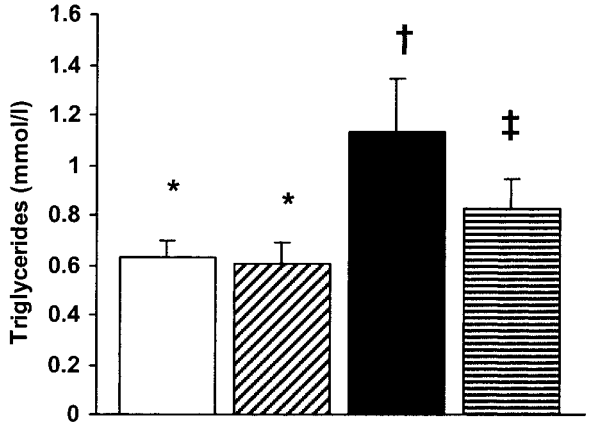

FIG. 3. Mean fasting triglyceride concentration after control condition (□), after 28 days of fish oil supplementation (▨), after 6 days of high-fructose diet (■), and after 28 days of fish oil supplementation plus high-fructose diet (▤) in seven men. Values are means ± SE represented by vertical bars. Values not sharing the same superscripts are significantly different ($P < 0.05$).

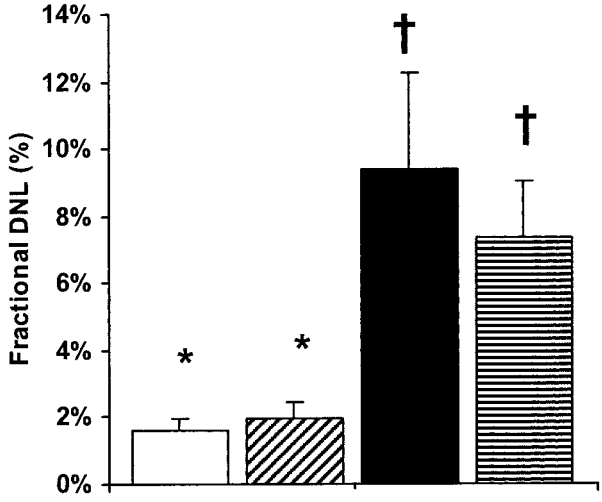

FIG. 4. Mean baseline fractional hepatic DNL after control condition (□), after 28 days of fish oil supplementation (▨), after 6 days of high-fructose diet (■), and after 28 days of fish oil supplementation plus high-fructose diet (▤) in seven men. Values are means ± SE represented by vertical bars. Values not sharing the same superscripts are significantly different ($P < 0.05$).

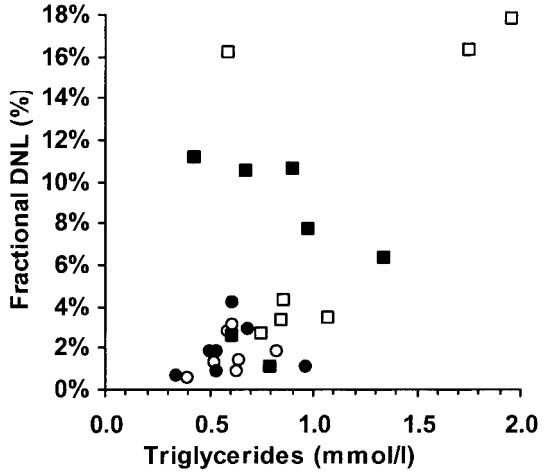

FIG. 5. Relationship between fractional hepatic DNL and triglyceride concentration after control condition (○), after 28 days of fish oil supplementation (●), after 6 days of high-fructose diet (□), and after fish oil supplementation plus high-fructose diet (■) in seven men.

plasma triglycerides in all four conditions is illustrated in Fig. 5.

Hyperinsulinemic-euglycemic clamp. Figure 6 shows plasma glucose and insulin concentrations as well as insulin resistance during hyperinsulinemic-euglycemic clamp. Glycemia was maintained at 4.9 ± 0.1 mmol/l and did not differ between the four conditions. Insulin concentrations and insulin resistance did not vary significantly between the four conditions. Similarly, glucose oxidation at $t = 180$ (control subjects, 16.0 ± 1.0; fish oil alone, 16.3 ± 1.3; high-fructose diet, 18.8 ± 0.6; high-fructose diet plus fish oil, 19.6 ± 0.4 μmol · kg^{-1} · min^{-1}) and lipid oxidation at $t = 180$ (control subjects, −0.09 ± 0.06; fish oil alone, −0.22 ± 0.08; high-fructose diet, −0.14 ± 0.10; high-fructose diet plus fish oil, −0.22 ± 0.06 mg · kg^{-1} · min^{-1}) did not differ significantly under all four conditions.

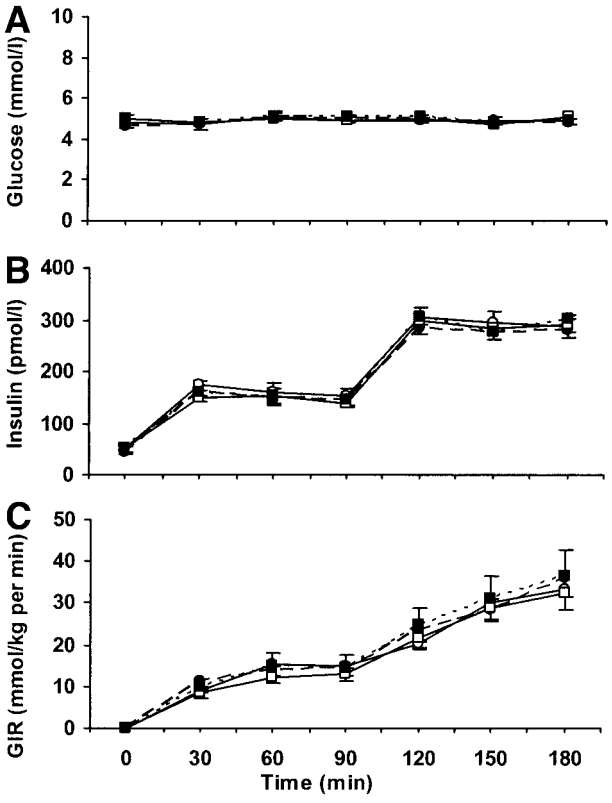

FIG. 6. Plasma glucose (*A*), insulin concentrations (*B*), and glucose infusion rate (*C*) during euglycemic-hyperinsulinemic clamping after control condition (○), after 28 days of fish oil supplementation (●), after 6 days of high-fructose diet (□), and after fish oil supplementation plus high-fructose diet (■) in seven men. Values are means ± SE represented by vertical bars.

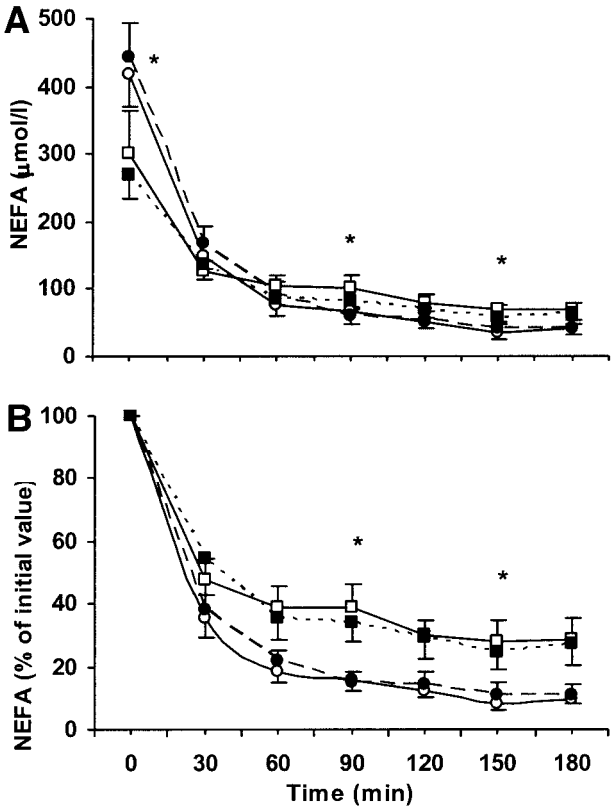

FIG. 7. NEFAs expressed as absolute values (*A*) and in percentage of the baseline value (*B*) during euglycemic-hyperinsulinemic clamping after control condition (○), after 28 days of fish oil supplementation (●), after 6 days of high-fructose diet (□), and after fish oil supplementation plus high-fructose diet (■) in seven men. Values are means ± SE represented by vertical bars. *$P < 0.05$ high-fructose diet versus control.

In Fig. 2, EGP is displayed in fasting condition and at $t = 90$ and $t = 180$ into the clamp. Although suppression of EGP in high-fructose diet (28% after $t = 180$) and high-fructose diet plus fish oil (24% after 180 min) were not significant, suppression in control subjects (44% at $t = 180$) and fish oil (36% at $t = 180$) were both significant ($P < 0.05$).

Figure 7 shows NEFA concentrations expressed as absolute values (Fig. 7*A*) and as percent of fasting values ($t = 0$) (Fig. 7*B*). During the clamp, NEFAs were higher after high-fructose diet compared with control subjects and fish oil alone. At times $t = 90$ and $t = 150$, differences between high-fructose diet and control subjects were significant ($P < 0.05$). Under high-fructose diet plus fish oil, NEFA concentrations tended to be lower compared with high-fructose diet alone throughout the clamp, but the difference did not reach statistical significance (Fig. 7*A*). In all four conditions, NEFA concentrations were significantly suppressed ($P < 0.01$) during the clamp.

However, NEFA suppression was significantly stronger ($P < 0.05$) after control subjects (90% at $t = 180$) and after fish oil alone (91% at $t = 180$) compared with high-fructose diet (76% at $t = 180$) and high-fructose diet plus fish oil (77% at $t = 180$) (Fig. 7*B*).

DISCUSSION

Fructose overfeeding for 6 days led to the development of several features of the metabolic syndrome in these healthy, normal-weight volunteers. First, fructose overfeeding significantly increased plasma triglycerides concentration by 79%. This increase was associated with a sixfold stimulation of fractional hepatic DNL. Although the kinetics of VLDL particles were not assessed, it is likely that stimulation of hepatic DNL by fructose contributed to the observed hypertriglyceridemia (Fig. 5). Previous studies have indeed observed significant correlations between stimulation of fractional hepatic DNL and plasma triglycerides levels in humans fed high simple carbohydrate diets (4,19). Second, fructose increased fasting glycemia and EGP and blunted suppression of EGP during the clamp experiment, indicating hepatic insulin resistance (Fig. 2). Similar observations have been made in rodents after 2 weeks of high-sucrose diet (20). In rodents, as in our study, this increase in EGP was not associated with fasting hyperinsulinemia, suggesting that hepatic insulin resistance alone does not stimulate insulin secretion. Third, and quite unexpectedly, fructose impaired the suppression of plasma NEFA during low and moderate insulin infusion rate, indicating adipose tissue insulin resistance (Fig. 7).

The mechanisms by which fructose exerts these various effects cannot be determined from the present experiments and can only be speculated. Changes in adiponectin and resistin concentrations could have been suspected given the effects of these adipokines on whole body and hepatic insulin resistance (21,22). However, their concentrations were not altered by high-fructose diet or fish oil.

Fructose overfeeding exposes the liver to a huge carbohydrate load. The fructose carbons can be converted into glucose and glycogen, thus leading to increased hepatic glycogen stores (23,24). This process may contribute to enhanced EGP and to its impaired suppression by insulin (25). The suppression of glucose production during a hyperinsulinemic clamp may, however, not truly reflect what occurs during oral feeding when the liver will be exposed to higher concentrations of glucose due to portal glucose delivery. A portion of fructose carbons can be also converted into lactate, leading to hyperlactatemia and possible extrahepatic metabolic effects in muscle and adipose tissue (26,27). Finally, carbon atoms can be converted into fatty acids, thus enhancing hepatic DNL (28–30). However, these acute effects of fructose administration are unlikely to account for our observation of increased EGP, DNL, triglyceridemia, and lactatemia after an overnight fast (i.e., more than 10 h after ingestion of fructose). These effects are most likely explained by alterations of hepatic gene expression by high-fructose diet (28). In this regard, it is documented that high carbohydrates diets stimulate carbohydrate response element–binding protein (ChREBP), a hepatic transcription factor that upregulates the expression of lipogenic and glycolytic enzymes (31). ChREBP also regulates the expression of key enzymes of hepatic fructose metabolism. Although ChREBP is primarily regulated by glucose through the increase in intrahepatic glucose metabolites, it may possibly be stimulated as well during high-fructose diet. In addition, recent findings show that hepatic stress may mediate hepatic insulin resistance associated to high-fructose diet (32,33).

Regarding the effects of fructose overfeeding on adipose tissue, we have no mechanistic explanation to offer. Although it is generally accepted that fructose is nearly completely metabolized at first pass in the gut and the liver (34), it remains possible that some fructose reached the systemic circulation and exerted metabolic effects directly at the level of adipocytes. Alternatively, fructose metabolism in the liver may possibly exert effects on adipose tissue through the release of metabolites such as lactate (24). It could also be speculated that fructose overfeeding activated the sympathetic nervous system, as it has been demonstrated in rodents (35) and hence stimulated lipolysis.

Despite the observed hepatic and adipose tissue insulin resistance, whole-body insulin-stimulated glucose disposal was not decreased after six days of fructose overfeeding. Because skeletal muscle glucose metabolism is the major determinant of whole-body glucose disposal under conditions of hyperinsulinemia (36), this strongly suggests that muscle insulin sensitivity was not impaired. Similar observations were made in rodents after 2 weeks of a diet rich in sucrose (20). Rodents, however, were shown to subsequently develop whole-body insulin resistance after several weeks of high-sucrose feeding (20), and it is therefore likely that the duration of fructose overfeeding was too short to produce effects in skeletal muscle.

We observed that fish oil supplementation significantly blunted fructose-induced hypertriglyceridemia. The hypotriglyceridemic effect of fish oil in animals (37) and humans (38) is well known. The mechanisms by which this effect was attained can only be speculated on the basis of our data. Although hepatic DNL was not significantly reduced after fish oil supplementation, we cannot exclude that the number of subjects studied was too small to detect such an effect. Alternatively, fish oil may have reduced the secretion of triglycerides-rich particles and/or stimulated their extrahepatic clearance (39,40). Whatever the mechanism, the hypotriglyceridemic effect of fish oil was not associated with a reduction of EGP or with improved insulin-induced NEFA suppression (Fig. 2 and 7), indicating that it failed to impact the metabolic steps involved in hepatic and adipose tissue insulin resistance. In rats however, fish oil has been shown to prevent whole body insulin resistance (6) after several weeks of high sucrose feeding (41). This suggests that fish oil could have the potential to prevent development of whole-body insulin resistance induced by chronic fructose overfeeding in humans.

Our study design involved administration of an extra amount of fructose while leaving the other dietary intakes unchanged. It therefore resulted in both energy and fructose overfeeding. As such, it is representative of a condition where increased dietary fructose intake would not be compensated by a reduction of calories from other sources. It cannot, however, truly differentiate the effects

of high-fructose intake per se and of energy total carbohydrate overfeeding. Only comparative studies involving subjects overfed with fructose versus starch or glucose will be able to address this issue.

In conclusion, we found a hepatic phenotype that was characterized by hepatic (and adipose tissue) insulin resistance, hypertriglyceridemia, and increased DNL after 6 days of fructose overfeeding. However, whole-body insulin-mediated glucose disposal remained unchanged, suggesting normal muscle insulin sensitivity. Experiments done in rodents, however, suggest that exposure to large amounts of fructose for several weeks may impair muscle insulin sensitivity as well. Despite its significant hypotriglyceridemic effect, fish oil supplementation did not improve the other metabolic alteration induced by fructose. Therefore, the overall consequences of fructose overfeeding over longer periods of time and the potential preventive properties of fish oil in the development of whole-body insulin resistance remain to be evaluated.

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A 4-wk high-fructose diet alters lipid metabolism without affecting insulin sensitivity or ectopic lipids in healthy humans[1–4]

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ABSTRACT

Background: High fructose consumption is suspected to be causally linked to the epidemics of obesity and metabolic disorders. In rodents, fructose leads to insulin resistance and ectopic lipid deposition. In humans, the effects of fructose on insulin sensitivity remain debated, whereas its effect on ectopic lipids has never been investigated.

Objective: We assessed the effect of moderate fructose supplementation on insulin sensitivity (IS) and ectopic lipids in healthy male volunteers ($n = 7$).

Design: IS, intrahepatocellular lipids (IHCL), and intramyocellular lipids (IMCL) were measured before and after 1 and 4 wk of a high-fructose diet containing 1.5 g fructose · kg body $wt^{-1} \cdot d^{-1}$. Adipose tissue IS was evaluated from nonesterified fatty acid suppression, hepatic IS from suppression of hepatic glucose output (6,6-$^2H_2$-glucose), and muscle IS from the whole-body glucose disposal rate during a 2-step hyperinsulinemic euglycemic clamp. IHCL and IMCL were measured by ^{1}H magnetic resonance spectroscopy.

Results: Fructose caused significant ($P < 0.05$) increases in fasting plasma concentrations of triacylglycerol (36%), VLDL-triacylglycerol (72%), lactate (49%), glucose (5.5%), and leptin (48%) without any significant changes in body weight, IHCL, IMCL, or IS. IHCL were negatively correlated with triacylglycerol after 4 wk of the high-fructose diet ($r = -0.78$, $P < 0.05$).

Conclusion: Moderate fructose supplementation over 4 wk increases plasma triacylglycerol and glucose concentrations without causing ectopic lipid deposition or insulin resistance in healthy humans. *Am J Clin Nutr* 2006;84:1374–9.

KEY WORDS Cardiovascular disease risk, dyslipidemia, healthy men, ectopic lipids, insulin sensitivity, fructose

INTRODUCTION

Over the past decades, fructose consumption per capita has dramatically increased, mainly because of a higher consumption of sugar-sweetened beverages (1). Furthermore, several authors have suggested that fructose (as either sucrose or high-fructose corn syrup) might play a role in the onset of metabolic disorders and excess weight gain (1, 2). This concern is supported by observations that, in rodents, a high-fructose diet (HFD) induces hepatic insulin resistance, increases intrahepatocellular lipids (IHCL), and stimulates hepatic de novo lipogenesis within a few days (3, 4). When sustained over longer periods of time, high fructose or sucrose intakes induce hepatic steatosis (5, 6) and whole-body insulin resistance with a concomitant accumulation of intramyocellular lipids (IMCL) (3, 7, 8). In humans, several studies have addressed the chronic effects of fructose ingestion on lipid and carbohydrate metabolism, but many issues remain unresolved. A high fructose intake has clearly been shown to increase plasma triacylglycerol concentrations (9–11). In contrast, the effect of high fructose intakes on insulin sensitivity is still debated. As recently reviewed, some authors have observed increased fasting glucose and insulin concentrations after high fructose consumption, whereas others have reported no effect on glucose homeostasis or markers of insulin sensitivity (12). No study to date had addressed the effects of dietary fructose on ectopic lipids.

In a previous study, we reported that a 6-d HFD induces dyslipidemia as well as hepatic and adipose tissue insulin resistance without altering whole-body insulin sensitivity (13). To further delineate the metabolic consequences of a longer period of high fructose consumption with a focus on insulin sensitivity, we studied a group of 7 healthy males submitted to a 4-wk HFD. The amount of daily fructose consumption added to the diet corresponded with the fructose content of 2 L of soda.

SUBJECTS AND METHODS

Subjects

Seven healthy, nonsmoking, white male volunteers (mean ± SEM age: 24.7 ± 1.3 y) took part in the study (**Table 1**). According to a physical examination and a brief medical history, all

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TABLE 1
Fasting anthropometric and metabolic variables[1]

	Baseline	High-fructose diet			
		1 wk	2 wk	3 wk	4 wk
Anthropometric variables					
Body weight (kg)	69.3 ± 2.6	69.3 ± 2.5	69.0 ± 2.6	69.3 ± 2.7	69.5 ± 2.7
Body fat (%)	17 ± 1	17 ± 1	16 ± 1	16 ± 1	16. ± 1
Mean blood pressure (mm Hg)	83 ± 2	83 ± 2	84 ± 1	85 ± 2	84 ± 1
Metabolic variables					
Glucose (mmol/L)	4.9 ± 0.1	5.0 ± 0.1	5.2 ± 0.1	4.9 ± 0.1	5.2 ± 0.1[2]
Lactate (mmol/L)	0.83 ± 0.05	0.97 ± 0.11	1.24 ± 0.11[2]	1.25 ± 0.06[2]	0.92 ± 0.04
Insulin (pmol/mL)	50.4 ± 3.6	57.6 ± 5.4	56.4 ± 5.4	58.8 ± 5.4	51.0 ± 2.4
Glucagon (pmol/L)	67 ± 9	67 ± 8	70 ± 10	70 ± 10	71 ± 11
Total triacylglycerols (mmol/L)	0.64 ± 0.05	0.87 ± 0.08[2]	0.83 ± 0.05[2]	0.76 ± 0.07	0.94 ± 0.06[2]
VLDL-triacylglycerols (mmol/L)	0.36 ± 0.04	0.62 ± 0.08[2]	0.58 ± 0.05[2]	0.53 ± 0.07[2]	0.70 ± 0.06[2]
Nonesterified fatty acids (μmol/L)	740 ± 92	488 ± 37[2]	368 ± 17[2]	378 ± 45	515 ± 39[2]
Cholesterol (mmol/L)	3.8 ± 0.3	3.8 ± 0.3	4.2 ± 0.3	4.2 ± 0.3	4.0 ± 0.3
β-Hydroxybutyrate (mmol/L)	0.24 ± 0.04	0.12 ± 0.04	0.06 ± 0.02[2]	0.06 ± 0.02[2]	0.05 ± 0.01[2]
Leptin (ng/mL)	2.1 ± 0.2	2.3 ± 0.2[2]	2.5 ± 0.3	3.3 ± 0.4[2]	3.2 ± 0.4[2]

[1] All values are $\bar{x}$ ± SEM; $n = 7$.
[2] Significantly different from baseline, $P < 0.05$ (Friedman's ANOVA and Page's test, followed by Wilcoxon's matched-pairs signed-ranks test).

subjects were in good health, with body mass indexes (in kg/m^2) between 19 and 25, and were moderately physically active (<1 h/wk). They were not taking any medications and did not regularly consume alcohol or sugar-sweetened beverages. The study was approved by the ethical committee of Lausanne University School of Medicine, and all participants provided written informed consent.

Study design and diet

During the initial 2 wk of the study, all subjects were thoroughly instructed by a trained dietitian to consume an isoenergetic diet containing ≈55% carbohydrates, 30% fat, and 15% protein. They were further instructed to consume a minimal amount of either sucrose-sweetened or artificially sweetened drinks and food during this period, with a resultant fructose consumption of <20 g/d. Thereafter, they were switched to a HFD consisting of the same isoenergetic diet with the addition of 1.5 g fructose · kg body wt^{-1} · d^{-1} for 4 wk. Fructose was consumed as a 20% solution with the 3 main meals and represented an excess of 18% of the subjects' daily energy requirement. The subjects' adherence to the prescribed fructose consumption was verified by collecting the empty fructose containers, and all subjects reported a 3-d dietary record before each test. Leisure-time sport activity was restricted to <1 h/wk throughout the study period. Fasting blood samples were collected weekly, and insulin sensitivity, IHCL, and IMCL were measured at baseline and after 1 and 4 wk of the HFD (**Figure 1**). Insulin sensitivity was determined by a two-step hyperinsulinemic euglycemic clamp. IHCL and IMCL contents were assessed by proton magnetic resonance spectroscopy (^{1}H-MRS).

Metabolic investigation

Subjects reported at 0700 to the metabolic unit of the Lausanne University Hospital after they had fasted for 12 h overnight. On arrival, the subjects were asked to void, and their body composition was estimated from subcutaneous skinfold-thickness measurements at the biceps, triceps, subscapular, and suprailiac sites (14). While the subjects rested quietly in a bed in a semirecumbent position, an indwelling catheter was inserted into the vein of the right wrist for blood sampling. A second indwelling catheter was inserted into an antecubital vein of the other arm for glucose, insulin, and tracer infusions. Whole-body glucose turnover was assessed in the basal condition after a 2-h 6,6-$^2H_2$ glucose infusion (bolus: 2 mg/kg; continuous: 20 μg · kg^{-1} · min^{-1}). Blood was collected at baseline for measurement of plasma concentrations of glucose, insulin, glucagon, leptin, lactate, nonesterified fatty acids (NEFAs), β-hydroxybutyric acid, triacylglycerol, total cholesterol, and VLDL, LDL, and HDL subfractions. Energy expenditure and substrate utilization were continuously measured by indirect calorimetry (ventilated canopy) from 0800 to 1300 (15) by using the equations of Livesey and Elia (16). At

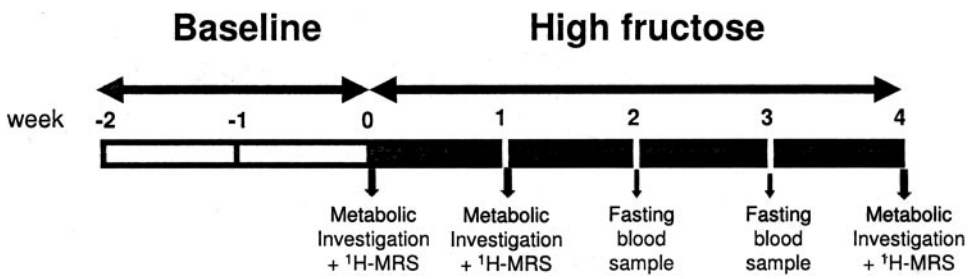

FIGURE 1. Experimental protocol. MRS, magnetic resonance spectroscopy.

baseline, heart rate and blood pressure were measured 3 times over a 20-min period.

Whole-body, liver, and adipose insulin sensitivity were measured for 3 h after the initial 2-h tracer infusion, from 1000 to 1300. A two-step, hyperinsulinemic, euglycemic clamp (0.3 and 1 mU · kg^{-1} · min^{-1}, 90 min each) (17, 18) was performed in combination with measures of hepatic glucose output (6,6-$^2H_2$ glucose, "hot infusion model") (19) and lipolysis (plasma NEFA concentrations). Blood samples were collected every 5 min during the clamp to monitor plasma glucose concentrations and at 30-min intervals for the analysis of tracer, insulin, glucagon, triacylglycerol, and NEFA concentrations.

Analytic procedures

Plasma was immediately separated from blood by centrifugation at 4 °C for 10 min at 3600 × *g* and was stored at −20 °C. Colorimetric methods were used to assess plasma concentrations of NEFAs (kit from Wako Chemicals, Freiburg, Germany) and triacylglycerol (kit from Biomérieux Vitek Inc, Lyon, France). Commercial radioimmunoassay kits were used for the determination of plasma insulin, glucagon, and leptin (LINCO Research, St Charles, MO). Subfractions of lipoproteins were separated by ultracentrifugation. β-Hydroxybutyric acid and lactate concentrations were measured enzymatically by using kits from Boehringer (Boehringer Mannheim, Mannheim, Germany). During the clamp, plasma glucose concentrations were measured by the glucose oxidase method with a Beckman glucose analyzer II (Beckman Instruments, Fullerton, CA). Plasma 6,6-$^2H_2$ glucose isotopic enrichment was measured by gas chromatography–mass spectrometry (Hewlett-Packard Instruments, Palo Alto, CA), as previously described (20).

^{1}H Magnetic resonance spectroscopy

All ^{1}H-MRS examinations were performed on a clinical 1.5 T MR scanner (Signa; General Electric Medical Systems, Waukesha WI) by using a transmit-receive extremity coil for the calf and a flexible receive coil in combination with the body transmit coil for the liver. Data acquisition and processing of spectra from the tibialis anterior followed the previously described protocol (21). In short: single-voxel ^{1}H-MR spectra were acquired with an optimized point-resolved spectroscopy (PRESS) sequence (repetition time = 3 s, echo time = 20 ms, 128 acquisitions, 16 phase rotating steps, 2-kHz bandwidth, 1024 points). The voxel in the calf measured 11 × 12 × 18 mm^3 in R/L×A/P×I/S direction and was placed in the tibialis anterior muscle of the right leg. The spectra were quantified after eddy current correction by using the fully relaxed unsuppressed water signal as internal concentration standard. All results are expressed as mmol/kg wet wt.

Acquisition of reproducible and reliable MR spectra from a volume in the liver is hampered by effects of respiratory and potentially cardiac motion. To reduce these influences, data acquisition was double-triggered to both respiration and the electrocardiogram by using a method developed for ^{1}H-MRS of the heart (22), which makes use of the fact that the electrocardiogram amplitude depends on respiratory motion and that allows us to restrict electrocardiogram-triggered data acquisition to periods of expiration. On the basis of axial MR images (spoiled gradient recalled echo sequence, 60° flip angle, 1.5-ms echo time, 0.11-s repetition time, 4-mm slice thickness, 1-mm gap between slices, 40-cm field of view, 512 × 192 matrix size) obtained in breath hold, a volume of interest of 4.3 cm^3 was placed in a lateral area of the liver and repositioned at the same location in follow-up examinations. MR spectra were recorded from this volume with the same short echo time PRESS sequence used for the investigation of the calf (echo time 20 ms). Sixty-four acquisitions with water presaturation were recorded and stored individually for each spectrum. Effects of residual motion were found to be evidenced by small shifts in resonance frequency. They were accounted for by realigning those individual scans in a frequency domain that fell into a bandwidth of 12 Hz and by discarding those acquisitions with a lipid peak shifted by more than ± 6Hz. Spectra were processed, fitted, and quantitated similarly to the muscle spectra. Quantitation to obtain IHCL in units of mmol/kg was based on the median water signal from 8 separate acquisitions obtained without water suppression, a T_2 of 50 ms for this water signal (as determined from separate acquisitions with varying echo time), and an assumed liver water content of 71.1%. Reproducibility of IMCL and IHCL determinations on immediate repetition of the measurement in completely independent examinations was established earlier and was found to be 6% for IMCL (23) and 10% for IHCL (R Kreis, P Vermathen, M Ith, K-A Lê, L Tappy, C Boesch, unpublished observations, 2006), whereas variations between healthy subjects were found to be much larger.

Statistical analysis

Throughout the manuscript, data are expressed as means ± SEMs. Statistical analyses were performed with STATA version 8.2 (Stata Corp, College Station, TX), and $P < 0.05$ was considered statistically significant. The significance of mean differences among conditions was determined with Friedman's analysis of variance (ANOVA) and Page's test (24). Post hoc comparisons were done by using the Wilcoxon's matched-pairs signed-ranks test. Relations between IHCL and IMCL and total triacylglycerol or VLDL-triacylglycerol were assessed by using Spearman's correlation test.

RESULTS

Body weight, body composition, and mean blood pressure were not significantly affected by the 4-wk HFD intervention. However, within one week, the HFD caused significant ($P < 0.05$) increases in fasting VLDL-triacylglycerol (72%), total triacylglycerols (36%), and leptin (48%), whereas lactate increased only after 2 wk (49%) and glucose after 4 wk (5.5%). Fasting NEFA and β-hydroxybutyrate concentrations decreased significantly after 1 and 2 wk of the HFD, respectively (NEFA: −34%; β-hydroxybutyrate: −75%). Fasting insulin, glucagon, and total cholesterol concentrations did not change significantly during the HFD ($P > 0.05$; Table 1 and **Figure 2**).

Despite no significant changes in energy expenditure and fasting hepatic glucose output over the 4-wk HFD treatment, carbohydrate oxidation increased after 1 wk (34%; $P < 0.05$) with a concomitant trend toward decreased lipid oxidation ($P = 0.09$; **Table 2**). IMCL and IHCL concentrations were unchanged by HFD treatment (Figure 2). There was a significant negative correlation between IHCL and fasting triacylglycerol after 4 wk of the HFD ($r = -0.78$, $P < 0.05$), whereas this correlation fell short of significance at baseline and after 1 wk ($r = -0.55$ and -0.54, respectively; $P > 0.05$). No significant correlation was observed between IMCL and total- or VLDL-triacylglycerol.

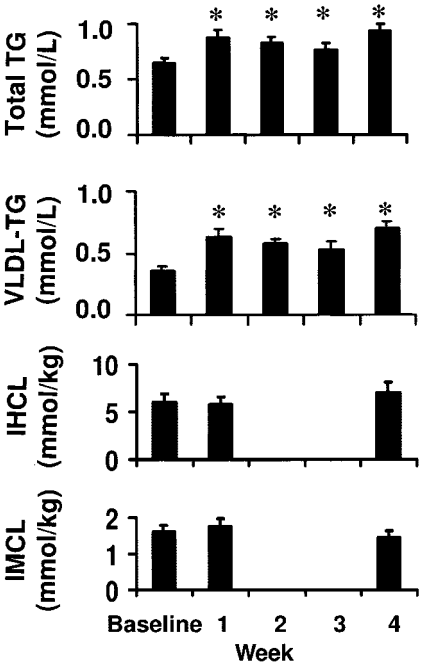

FIGURE 2. Mean (±SEM) plasma triacylglycerol (TG), VLDL-TG, intrahepatocellular lipids (IHCL), and intramyocellular lipids (IMCL) in response to the high-fructose diet. $n = 7$. *Significantly different from baseline, $P < 0.05$ (Friedman's ANOVA and Page's test, followed by Wilcoxon's matched-pairs signed-ranks test).

During the hyperinsulinemic clamp studies, plasma glucose concentrations were successfully maintained at 5.5 ± 0.1 mmol/L. During the baseline clamp procedure, insulin concentrations were 222 ± 42 pmol/L during the first step and increased to 510 ± 12 pmol/L during the second step of the clamp, reaching similar plateaus after 1 and 4 wk of the HFD. The suppression of hepatic glucose output by the low-dose insulin infusion ($t = 90$ min) was not significantly affected by the HFD treatment. Similarly, the glucose disposal rate, glucose and lipid oxidation rates, and the nonoxidative glucose disposal rate ($t = 180$ min) were not significantly affected by the HFD treatment (Table 2).

TABLE 2
Metabolic variables under basal and hyperinsulinemic (T90, low insulin infusion; T180, high insulin infusion) conditions[1]

		High-fructose diet	
	Baseline	1 wk	4 wk
Hepatic glucose output (μmol · kg^{-1} · min^{-1})			
Baseline	13.3 ± 0.5	13.9 ± 0.5	13.9 ± 0.5
T90	6.1 ± 0.5	7.8 ± 0.5	6.1 ± 1.1
T180	3.9 ± 2.2	2.2 ± 2.2	1.7 ± 2.2
Glucose disposal rate (μmol · kg^{-1} · min^{-1})			
T90	18.3 ± 0.5	20.6 ± 0.5	19.4 ± 1.1
T180	28.3 ± 3.4	30.0 ± 4.4	28.3 ± 4.4
Carbohydrate oxidation (μmol · kg^{-1} · min^{-1})			
Baseline	8.3 ± 1.7	11.1 ± 1.1[2]	9.5 ± 1.7
T90	11.1 ± 1.1	10.6 ± 1.1	12.8 ± 2.2
T180	13.9 ± 1.1	18.9 ± 1.7[2]	17.2 ± 1.7
Nonoxidized carbohydrates (μmol · kg^{-1} · min^{-1})			
Baseline	5.0 ± 1.7	3.3 ± 1.7	5.0 ± 2.2
T90	7.2 ± 0.5	10.0 ± 1.1[2]	7.2 ± 2.2
T180	16.1 ± 3.9	13.3 ± 5.0	12.8 ± 5.6
Lipid oxidation (mg · kg^{-1} · min^{-1})			
Baseline	0.7 ± 0.2	0.4 ± 0.2	0.5 ± 0.1
T90	0.4 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
T180	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1[2]
Energy expenditure (kcal/min)			
Baseline	1.02 ± 0.04	0.98 ± 0.02	0.98 ± 0.03
T180	1.04 ± 1.01	1.01 ± 0.02	1.03 ± 0.03

[1] All data are $\bar{x}$ ± SEM; $n = 7$.
[2] Significantly different from baseline, $P < 0.05$ (Wilcoxon's matched-pairs signed-ranks test).

DISCUSSION

A 4-wk fructose supplementation (1.5 g · kg body wt^{-1} · d^{-1}) induced moderate but sustained increases in plasma triacylglycerol, VLDL-triacylglycerol, and leptin paralleled by a modest increase in fasting plasma glucose without any significant change in body weight. In contrast, hepatic, adipose, and whole-body insulin sensitivity as well as liver and muscle lipid contents were unchanged by the 4-wk fructose supplementation.

The increase in fasting VLDL-triacylglycerol observed in the present study corroborates several previous reports in humans (10, 11, 25) and animals (26). Both a stimulation of hepatic VLDL-triacylglycerol synthesis and secretion and decreased VLDL-triacylglycerol clearance may be involved in this process (27).

In a previous study, we reported that fructose supplementation at twice the dose used in the present study led to an 80% increase in plasma VLDL-triacylglycerol (13). Our present findings further extend this observation by showing that this effect, first, is dependent on the dose of fructose administered, and second, is sustained for ≥4 wk. Given the epidemiologic association observed between plasma total or VLDL-triacylglycerol concentrations and atherosclerotic vascular disorders (28), these observations strongly suggest that consumption of even modest amounts of fructose may significantly increase the risk of development of cardiovascular disease.

We also observed a small but significant increase in fasting glycemia after 4 wk of the HFD. In our previous study, supplementation with twice the dose of fructose used in this study led to significant increases in fasting glycemia and hepatic glucose output (13). Our present results failed to reproduce the increase in hepatic glucose output, probably because of the lower fructose intake. However, our observation of a higher fasting plasma glucose concentration still suggests some degree of impairment in the inhibition of glucose production by glycemia (29, 30).

These results contrast with those obtained in rodents submitted to a high-sucrose diet. After sucrose feeding, rodents develop early hepatic insulin resistance and increased IHCL. High sucrose intake eventually leads to the development of whole-body insulin resistance with a concomitant increase in IMCL after 5 to 6 wk of treatment (3). The fructose part of sucrose appears to be the major culprit for these effects (31). This time course in the development of insulin resistance suggests a central role of the liver before the development of whole-body insulin resistance

(32). The metabolic events observed in our study differ markedly from these animal studies. First, neither IHCL nor IMCL increased at any time; second, except for the modest increase in glycemia observed after 4 wk, there was no evidence for a reduction in hepatic or whole-body insulin sensitivity. Several explanations may account for these differences. The relative doses of fructose consumed in these animal experiments were much larger than in the present study. It has also been reported that, in rodents, lower sucrose intake as well leads to the development of insulin resistance, although with a longer delay (33). It is therefore possible that such modest doses of fructose would require a longer exposure to induce adverse effects on insulin sensitivity.

The absence of any change in IHCL and IMCL despite a sustained increase in plasma triacylglycerol and VLDL-triacylglycerol was unexpected and is most likely related to the absence of insulin resistance after the HFD. Fructose, by increasing de novo lipogenesis, may enhance hepatic triacylglycerol production. These newly formed hepatic triacylglycerols can in turn be secreted as VLDL-triacylglycerol, stored as IHCL, or oxidized. If hepatic triacylglycerol synthesis were to determine both VLDL-triacylglycerol concentration and IHCL deposition, one would expect a positive correlation between IHCL and VLDL-triacylglycerol after fructose-induced stimulation of triacylglycerol synthesis. Our results are clearly at odds with this scenario. We observed a significant negative correlation between IHCL and total or VLDL-triacylglycerol after 4 wk of the HFD. This indicates that the HFD increased plasma triacylglycerol without altering IHCL, and this observation supports the hypothesis that exportation of newly formed triacylglycerol as VLDL-triacylglycerol is a key element to prevent IHCL accumulation. The HFD failed to increase not only IHCL but also IMCL and failed to alter whole-body insulin sensitivity. Therefore, we postulate that the extra amount of triacylglycerol formed from fructose was essentially secreted as VLDL, thus preventing ectopic fat deposition in the liver.

Finally, high fructose feeding led to a continuous rise in fasting plasma leptin concentrations. This observation is consistent with experiments performed on isolated adipocytes showing that fructose and glycolytic substrates and metabolites increase leptin secretion (34) and with studies performed in rats fed a high-fructose diet (35). In the present study, both fructose-induced hyperlactatemia and hypertriglyceridemia may therefore have contributed to stimulating leptin secretion by adipocytes. The increased leptin concentration may in turn account for the absence of body weight gain despite the substantial fructose energy load added to the diet. Indeed, it is possible that a reduction of nonfructose nutrients occurred, in which hyperleptinemia may play a role.

We want to stress that our study has several limitations that must be kept in mind when interpreting the results. First, because it was performed as an outpatient study, it was not possible to assess compliance with the dietary prescription and fructose supplementation. However, the consistent increases in plasma triacylglycerol concentrations and basal carbohydrate oxidation observed after the HFD make us confident that the bulk of fructose was indeed consumed. Second, the study was designed as an uncontrolled study and included a baseline with a low-fructose diet followed by an isocaloric diet with fructose supplementation. Because no comparison was made with a glucose-supplemented diet, it remains possible that the effects observed were due to the increased carbohydrate intake rather than to specific effects of fructose. Furthermore, the study design did not allow us to evaluate whether additional effects would have been observed if the same amount of fructose had been consumed as sucrose. However, several studies reported similar deleterious effects of both fructose and sucrose on lipid metabolism in humans when compared with glucose (10, 11, 36). Our study was also limited to a small group of healthy, young male volunteers. Whether the same results would have been observed in females, in older individuals, or in overweight or obese subjects clearly awaits further studies.

In conclusion, we showed that in healthy subjects, consumption of moderate amounts of fructose for 4 wk produced a sustained increase in fasting VLDL-triacylglycerol and a modest but significant rise in fasting glycemia. This increase in plasma VLDL-triacylglycerol, observed with a dietary fructose intake commonly encountered in westernized countries, may increase cardiovascular risk over the long term. Insulin resistance and ectopic fat deposition were, however, not observed. This leads us to propose that some of the deleterious effects of fructose may possibly be prevented in healthy subjects by adaptive metabolic changes in hepatic cells, skeletal muscle, or adipose tissue. Further studies will be required to evaluate the responses to HFD in subgroups of individuals with increased metabolic risk (such as offspring of patients with type 2 diabetes and overweight or obese patients) and the long-term consequences of a HFD in healthy subjects.

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K-AL, DF, RS, and LT designed the study and performed the clamp experiments. MI, RK, PV, and CB performed the MR measurements. K-AL, DF, ER, and LT analyzed the data. K-AL, DF, and LT wrote the draft manuscript with contributions and critical reviewing from ER, RS, MI, RK, PV, and CB. All the authors read, commented on, and contributed to the submitted and revised manuscript. None of the authors had a conflict of interest.

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Short Report

Effects of four-week high-fructose diet on gene expression in skeletal muscle of healthy men

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Abstract

Aims. – A high-fructose diet (HFrD) may play a role in the obesity and metabolic disorders epidemic. In rodents, HFrD leads to insulin resistance and ectopic lipid deposition. In healthy humans, a four-week HFrD alters lipid homoeostasis, but does not affect insulin sensitivity or intramyocellular lipids (IMCL). The aim of this study was to investigate whether fructose may induce early molecular changes in skeletal muscle prior to the development of whole-body insulin resistance.

Methods. – Muscle biopsies were taken from five healthy men who had participated in a previous four-week HFrD study, during which insulin sensitivity (hyperinsulinaemic euglycaemic clamp), and intrahepatocellular lipids and IMCL were assessed before and after HFrD. The mRNA concentrations of 16 genes involved in lipid and carbohydrate metabolism were quantified before and after HFrD by real-time quantitative PCR.

Results. – HFrD significantly ($P < 0.05$) increased stearoyl-CoA desaturase-1 (SCD-1) (+50%). Glucose transporter-4 (GLUT-4) decreased by 27% and acetyl-CoA carboxylase-2 decreased by 48%. A trend toward decreased peroxisomal proliferator-activated receptor-γ coactivator-1α (*PGC*-1α) was observed (−26%, $P = 0.06$). All other genes showed no significant changes.

Conclusion. – HFrD led to alterations of SCD-1, GLUT-4 and *PGC*-1α, which may be early markers of insulin resistance.

Résumé

Effets d'un régime riche en fructose durant quatre semaines sur l'expression génique dans le muscle squelettique chez l'homme..

Objectifs. – Une alimentation riche en fructose pourrait jouer un rôle dans l'augmentation de la prévalence de l'obésité et autres troubles métaboliques. Chez l'être humain, un régime riche en fructose (HFrD) d'une durée de quatre semaines perturbe l'homéostasie lipidique, cela sans toutefois induire une résistance à l'insuline ou une accumulation de lipides intramyocellulaires (IMCL). Le but de cette étude était d'examiner si le fructose modifie l'expression génique dans le muscle squelettique, avant qu'une résistance à l'insuline ne soit observable au niveau macroscopique.

Méthodes. – Des biopsies musculaires ont été réalisées chez cinq volontaires sains de sexe masculin, ayant précédemment participé à une étude impliquant un HFrD durant quatre semaines. Lors de cette étude, leur sensibilité à l'insuline (clamp hyperinsulinémique euglycémique) et leur concentration d'IMCL ont été mesurées. Les concentrations de mRNA de 16 gènes impliqués dans le métabolisme des lipides et glucides ont été mesurées également avant et après HFrD.

Résultats. – Le HFrD a augmenté de manière significative ($P < 0{,}05$) le *stearoyl-CoA-desaturase-1* (SCD-1) (+50 %). Le transporteur au glucose-4 (GLUT-4) a diminué de 27 % et *e'acetyl-CoA-carboxylase-2* de 48 %. Une tendance à une baisse du *peroxysomal-proliferator activated-receptor-γ-coactivator 1α* (*PGC*-1α) a été observée (−26 %, $P = 0{,}06$). Les autres gènes sont demeurés inchangés.

Conclusion. – Le fructose modifie l'expression génique de SCD-1 et GLUT-4, ce qui pourrait constituer des marqueurs précoces de la résistance à l'insuline.

Keywords: Fructose; Gene expression; Skeletal muscle; Insulin resistance

Mots clés : Fructose ; Expression génique ; Muscle squelettique ; Résistance à l'insuline

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Over the past decades, fructose consumption has dramatically increased and several authors have suggested that fructose may play a role in the onset of metabolic disorders [1,2]. In rodents, a high-fructose diet (HFrD) induces accumulation of intrahepatocellular (IHCL) and intramyocellular (IMCL) lipids, together with hepatic and muscle insulin resistance (IR) [3]. In healthy humans, we recently showed that HFrD increased fasting plasma triglycerides (TG), very low-density lipoprotein (VLDL)-TG, leptin and glucose [4,5]. Despite a sustained increase in VLDL-TG secretion, HFrD did not affect ectopic lipids or insulin sensitivity (hepatic/muscle). One possible explanation is that the deleterious effects of HFrD may not be detectable at the whole-body level, but that subtle molecular changes may be occurring in peripheral tissues. To evaluate this, we measured the changes in expression of selected genes involved in lipid, carbohydrate and energy metabolism in muscle biopsies taken from five subjects who had participated in a four-week HFrD study [5].

1. Research design and methods

Skeletal muscle biopsies were obtained from five healthy, non-smoking, Caucasian men, who had participated in a primary clinical study [5]. During the initial two weeks, they were instructed to consume an isoenergetic diet, with a minimal amount of artificially sucrose-sweetened drinks and food (< 20 g/day). Thereafter, they were switched to an HFrD consisting of the same isoenergetic diet, with additional 1.5 g fructose/kg body weight/day for four weeks (18% excess energy requirement). Insulin sensitivity (two-step euglycaemic hyperinsulinaemic clamp; insulin infusion rates: 0.3 and 1.0 mU/kg/min, 90 min each, together with 6,6-$^2H_2$ glucose) and ectopic lipids (proton magnetic resonance spectroscopy) were measured before and after HFrD, as previously described [5]. A muscle biopsy was obtained from vastus lateralis muscle immediately at the end of the clamp. The study was approved by the ethics committee of Lausanne University's School of Medicine.

The real-time quantitative PCR assay for mRNA has been previously described and validated [6]. Hypoxanthine phosphoribosyltransferase (HPRT) mRNA was measured by real-time quantitative PCR as a reference gene and levels of mRNA were expressed as a percentage ratio referred to the expression of HPRT. Gene expression of selected genes involved in lipid, carbohydrate and energy metabolism were measured. The complete list is shown in Table 1.

Comparisons between values before and after HFrD were assessed using the Wilcoxon matched-pairs signed-ranks test.

2. Results

The effects of fructose on hormones and substrate concentrations have been reported in detail in a previous publication [5]. However, as muscle biopsies were obtained from only five of the seven subjects included in the initial study, only the metabolic parameters of these five are shown in this section. Fructose caused significant ($P < 0.05$) increases in fasting plasma concentrations of TG (0.63 ± 0.08 vs. 0.98 ± 0.08 mmol/L, +55%), VLDL-TG (0.37 ± 0.06 vs. 0.78 ± 0.05 mmol/L, +110%), glucose (4.9 ± 0.1 vs. 5.1 ± 0.1 mmol/L, +4%) and leptin (2.0 ± 0.2 vs. 3.2 ± 0.6 ng/mL, +58%), with no changes in body weight (68 ± 4 vs. 68 ± 4 kg), IHCL (6.1 ± 1.2 vs. 6.3 ± 1.4 mmol/kg), IMCL (1.4 ± 0.2 vs. 1.5 ± 0.2 mmol/kg), and insulin-mediated glucose disposal at both low (3.5 ± 0.1 vs. 3.7 ± 0.2 mg/kg/min) and high (5.6 ± 0.7 vs. 6.2 ± 0.8 mg/kg/min) insulin concentrations.

Table 1
Gene expression in skeletal muscle from healthy subjects before and after four weeks of a high-fructose diet at the end of a three-hour euglycaemic hyperinsulinaemic clamp[a]

Gene name	Gene symbol	Baseline	High fructose	*P* value
Lipid metabolism				
Fatty acid translocase	FAT/CD36	21 ± 3	19 ± 4	1.0
Lipoprotein lipase	LPL	88 ± 29	71 ± 9	0.7
Cytosolic fatty acid-binding protein-3	FABP-3	49 ± 13	46 ± 20	0.6
Acyl-CoA synthetase long-chain-5	ACSL-5	3.8 ± 0.8	3.7 ± 0.6	0.5
Stearoyl-CoA desaturase-1	SCD-1	0.04 ± 0.01	0.08 ± 0.01[b]	0.02
Diacylglycerol O-acyltransferase	DGAT	1.6 ± 0.1	1.5 ± 0.2	0.6
Adipose differentiation-related protein	ADFP	1.0 ± 0.1	0.8 ± 0.1	0.6
Sterol regulatory element-binding protein	SREBP-1c	49 ± 2	53 ± 7	0.7
Carnitine palmitoyl-CoA transferase-1	CPT-I	193 ± 28	155 ± 22	0.14
Acetyl-CoA carboxylase-2	ACC-2	23 ± 4	12 ± 1[b]	0.02
Malonyl-CoA decarboxylase	MCD	1.9 ± 0.2	1.2 ± 0.2	0.11
Glucose metabolism				
Glucose transporter, type 4	GLUT-4	26 ± 3	19 ± 3[b]	0.02
Hexokinase-2	HK-2	98 ± 17	157 ± 25	0.14
Pyruvate dehydrogenase kinase-4	PDK-4	575 ± 136	460 ± 217	0.4
Energy metabolism				
Uncoupling protein-3	UCP-3	511 ± 86	393 ± 69	0.14
Peroxisomal proliferator-activated receptor-γ coactivator-1α	*PGC*-1 α	195 ± 26	144 ± 16	0.06

[a] Data are expressed as a percentage ratio referring to expression of hypoxanthine phosphoribosyltransferase (HPRT). Values are means ± SEM, $n = 5$.
[b] Significantly different vs. baseline ($P < 0.05$; by Wilcoxon matched-pairs signed-ranks test).

Gene expression is shown in Table 1. Fructose significantly ($P < 0.05$) increased stearoyl-CoA desaturase-1 (SCD-1) (+50%). HFrD also significantly decreased glucose transporter-4 (GLUT-4) (−27%) and acetyl-CoA decarboxylase-2 (ACC-2) (−48%). A trend toward a decreased expression of peroxisomal proliferator-activated receptor-γ coactivator-1α (*PGC*-1α) was observed (−26%, $P = 0.06$). Other investigated genes showed no significant changes.

3. Discussion

As reported in our primary study, the five subjects from whom muscle biopsies were obtained after four weeks of HFrD had increased fasting plasma VLDL-TG, glucose and leptin, but neither IR nor ectopic lipid deposition was observed [5]. Given the well-documented association between HFrD and IR in rodents [3], we suspected that major alterations following HFrD may not yet be detectable at the whole-body level after a four-week period, but that subtle molecular changes may be occurring in skeletal muscle. To evaluate this, we monitored the expression of 16 genes in skeletal muscle coding for key proteins involved in lipid, carbohydrate and energy metabolism.

Although most monitored genes remained unchanged, the expression of four relevant genes was altered by HFrD. We first observed a two-fold increase in the expression of SCD-1, a major lipogenic enzyme and key controller in the process of lipid partitioning [7]. High SCD-1 expression in mouse liver is associated with hepatic steatosis and IR [8], while treatment with SCD-1 antisense oligonucleotides, leading to an 80% reduction of SCD-1 in the rat liver, reverses a high-fat diet-induced hepatic IR [9]. In skeletal muscle, SCD-1 expression is increased in morbidly obese humans, and is associated with low rates of fatty-acid oxidation and increased monounsaturated fatty-acid concentrations [10]. These observations suggest that increased expression of SCD-1 may result in abnormal lipid partitioning, thus favouring ectopic lipid deposition. In our study, we observed a sustained increase in plasma TG and VLDL-TG, with no significant changes in IMCL after four weeks [5]. However, the rise in SCD-1 may reflect early fructose-induced molecular changes that may, in the long term, favour lipid deposition in skeletal muscle and lead to IR [11].

In addition, HFrD decreased insulin-stimulated GLUT-4 expression by 27%. GLUT-4 expression is induced by insulin, and the effect is blunted in non-diabetic obese individuals and in type 2 diabetic patients [6]. Although whole-body glucose uptake was not altered by HFrD, the reduction in GLUT-4 expression may also represent a first step toward IR.

We also observed a trend towards a decreased expression of *PGC*-1α, a major regulator of mitochondrial biogenesis and thermogenesis. Mitochondrial dysfunction is commonly observed in IR subjects [12]. Moreover, expression of *PGC*-1α and genes under its control is down-regulated in type 2 diabetes [13]. A decreased *PGC*-1α expression may, therefore, indicate a fructose-induced alteration of mitochondrial function that may eventually contribute to IR in the long term. Moreover, there was a trend towards a decrease in carnitine palmitoyl-transferase-I (CPT-I) and malonyl-CoA decarboxylase (MCD), which may be related to the observed decrease in lipid oxidation after fructose [5]. The latter point, however, remains speculative, as the trend towards a decrease in MCD was not mirrored by an increase in ACC-2 expression, as is usually observed in conditions associated with inhibition of lipid oxidation.

Subjects were studied under isocaloric conditions and after fructose supplementation, which suggests an increase in both total calories and the relative contribution of simple carbohydrates to total energy expenditure. However, the fact that our study did not include appropriate control groups with matched calorie/carbohydrate intake is a weakness that has to be acknowledged. Nevertheless, fructose had no detectable effects on body weight and body composition, which suggests that it was at least partly compensated for by a reduction in food intake. The increase in leptin observed after fructose [5] may have been instrumental in this compensation. Furthermore, such a reduction would not have been properly identified as the study was carried out on an outpatients basis with no accurate monitoring of food intake. Based on these considerations, we believe it unlikely that the effects observed were due to an increase in total energy intake. It remains nonetheless possible that the high simple-carbohydrate intake, rather than fructose per se, was responsible for these effects. Further studies comparing higher intakes of fructose versus glucose may help to answer these questions.

We conclude that a four-week HFrD increased the expression of SCD-1, and decreased the expression of GLUT-4, ACC-2 and *PGC*-1α in skeletal muscle. Although other metabolic genes remained unchanged, these alterations may represent early molecular markers for dietary fructose-induced IR in skeletal muscle.

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