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ORIGINAL ARTICLE

Effect of chronic coffee consumption on weight gain and glycaemia in a mouse model of obesity and type 2 diabetes

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OBJECTIVE: Epidemiological evidence shows that chronic coffee consumption in humans is correlated with a lower incidence of type 2 diabetes mellitus. For the experimental exploration of the underlying mechanisms, this effect needs to be replicated in an animal model of type 2 diabetes with a short lifespan.

DESIGN: Male C57BL/6 mice consumed regular coffee or water *ad libitum* and the development of obesity and diabetes caused by high-fat diet (55% lipids, HFD) was observed from week 10 on for 35 weeks in comparison with mice feeding on a defined normal diet (9% lipids, ND).

RESULTS: The massive weight gain in HFD mice was dose-dependently retarded ($P = 0.034$), the moderate weight gain in ND mice was abolished ($P < 0.001$) by coffee consumption, probably because of a lower feeding efficiency. The consumption of fluid (water or coffee) was significantly diminished by HFD ($P < 0.001$), resulting in a higher coffee exposure of ND mice. On week 21 intraperitoneal glucose tolerance tests (IPGTT) showed a dose-dependent faster decline of elevated glucose levels in coffee-consuming HFD mice ($P = 0.016$), but not in ND mice. Remarkably, a spontaneous decrease in non-fasting glycaemia occurred after week 21 in all treatment groups ($P < 0.001$). On week 39 the IPGTT showed diminished peak of glucose levels in coffee-consuming HFD mice ($P < 0.05$). HFD mice were hyperinsulinaemic and had significantly ($P < 0.001$) enlarged islets. Coffee consumption did not affect islet size or parameters of beta-cell apoptosis, proliferation and insulin granule content.

CONCLUSION: Coffee consumption retarded weight gain and improved glucose tolerance in a mouse model of type 2 diabetes and corresponding controls. This gives rise to the expectation that further insight into the mechanism of the diabetes-preventive effect of coffee consumption in humans may be gained by this approach.

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INTRODUCTION

In 2002, data from the Hoorn study suggested an inverse correlation between chronic coffee consumption and the incidence of type 2 diabetes.[1] This observation was regarded paradoxical, as acute administration of caffeine leads to the deterioration of the glucose tolerance.[2–4] The results of the Hoorn study were confirmed and extended by data from the Nurses health study and the Health professionals' follow-up study.[5]

These results have been questioned, since there is a number of potential confounders[6,7] and some conflicting epidemiological lines of evidence.[8,9] However, the obvious discrepancy between the acute deterioration of glucose tolerance and the long-term diabetes-preventive effect became at least partially understandable by the observation that the glucose increase during the oral glucose tolerance test was lower in habitual coffee drinkers than in non-drinkers.[10,11] Since then the weight of epidemiological evidence has developed clearly in favour of the diabetes-preventive effect of coffee consumption. Apparently, the association does not depend on race, gender or geographic distribution of the study populations.[12,13]

It remains unclear, however, by which mechanism(s) the diabetes-preventive effect is brought about. There is evidence for both an increased insulin secretory responsiveness and an increased insulin sensitivity.[14] Some but not all studies have observed a protective effect of decaffeinated coffee, pointing to a relevant role of coffee constituents other than caffeine, such as chlorogenic acid, which is a major source of antioxidants in the westernized diet.[15–18] Upon repeated exposure to caffeine, a tolerance develops to its adrenaline-releasing effect,[19] which is probably the mechanism responsible for the deterioration of glucose tolerance upon acute exposure. This habituation may also explain why chronic coffee consumption does not increase the risk of cardiovascular mortality in the general population and may even decrease it in patients with type 2 diabetes.[20]

In view of the overall safety profile of coffee consumption,[21,22] a rational use of the diabetes-preventive property seems feasible once the underlying mechanisms of action are better understood. In particular, the identification of the relevant coffee ingredients would profit from investigations in an animal model of type 2 diabetes. This requires the demonstration of the main characteristics of the epidemiological findings in these animals. Monogenetic diabetes models (for example, db/db mice) offer the advantage that the course of the disease is well predictable; however, the relevance of such models for the pathogenesis of human type 2 diabetes is debatable.[23] C57Bl/6 mice when fed a high-fat diet (HFD) develop obesity, glucose intolerance, hyperinsulinaemia and later on a fasting hyperglycaemia.[24,25] The metabolic disturbances remain less severe than in db/db mice; thus, the syndrome corresponds more to obesity-induced impaired glucose tolerance than to full-blown type 2 diabetes.

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As the effect in humans is the result of a decade-long coffee consumption, it was decided to expose these mice to coffee for a proportionally similar length of time, that is, 35 weeks, which corresponds to about 30% of the mean lifespan of C57BL/6 mice.[26] The present study shows that chronic consumption of regular coffee has significant diminishing effects on the body weight gain and on the blood glucose excursions during glucose tolerance tests.

MATERIALS AND METHODS

Animals

Male C57BL/6NCrl mice were obtained from Charles River Laboratories, Sulzfeld, Germany and female C57BL/6 J OlaHsd mice were from Harlan Winkelmann GmbH, Borchen, Germany. Artificial lighting was kept on a 12:12-h light–dark cycle. Room temperature was constant at 21 °C. Ethics approval was obtained from the regional authority for animal welfare (LAVES, Oldenburg, Germany). The male mice were kept individually to prevent status fighting after return from testing in the metabolic cage. Each treatment group as defined by the type of diet and the coffee exposure consisted of five animals.

Diet composition and coffee exposure

Mice were fed either a normal maintenance diet (V1534-0 R/M-H; Ssniff Spezialdiäten GmbH, Soest, Germany) containing 16.3 MJ kg^{-1} gross energy or 12.9 MJ kg^{-1} (3.1 kcal g^{-1}) metabolizable energy from 33% crude protein, 9% crude lipids and 58% crude carbohydrates (normal diet (ND)) or a HFD. The HFD (E15116-34; Ssniff Spezialdiäten GmbH) contained 24 MJ kg^{-1} gross energy and 20.6 MJ kg^{-1} (4.91 kcal g^{-1}) metabolizable energy from 17% crude protein, 55% crude lipids and 28% crude carbohydrates. The ND composition was designed to serve as control for the HFD. Roasted and ground coffee was brewed and paper-filtered at strengths of 20 and 40 g l^{-1}, that is, normal and high-strength coffee of human consumption.

Body weight, fluid and food intake

Food and fluid intake was *ad libitum*. At four time points (weeks 8, 18, 26 and 35) the individual food and fluid intake of C57BL/6NCrl mice was determined during an overnight stay (16 ± 0.5 h) in a wire-bottomed metabolic cage (model 2004, Tecniplast, Hohenpeißenberg, Germany). Food intake was measured by weighing the food given and the food remaining and correcting for the food spillage.[27] Faecal weight and urine volume were also measured.

Blood glucose measurement

An overnight fast to enable the measurement of fasting blood glucose interfered with the weight gain; thus, weekly blood glucose measurements were performed at the end of the light phase (ca 4 p.m.), that is, after a period with low spontaneous food intake. The glucose concentration in whole blood (1 μl obtained by tail bleeding) was measured in duplicate using a Free Style Lite glucometer (Abbott, Wiesbaden, Germany). Compared with serum glucose whole-blood glucose values are lower by ~15%.

IPGTT, insulin and HbA1c

The intraperitoneal glucose tolerance test (IPGTT) was performed after a fasting period of 6 h. A fixed dose of 50 mg glucose was injected as a prewarmed sterile-filtered solution in a volume of 200 μl Krebs–Ringer buffer. These conditions have been described to yield reliable values.[28] Blood was sampled at 0, 15, 30, 60 and 120 min by tail bleeding. Blood glucose was determined as described above. At 60 min, 30 μl blood was collected, immediately centrifuged (2000 *g* × 15 min) and the insulin content determined in 5 μl plasma using an ultrasensitive ELISA (Mercodia, Uppsala, Sweden). HbA1c was determined in 5 μl capillary blood using an immunoassay based on the boronate affinity technology (Nycocard, Axis-Shield, Heidelberg, Germany).

Lipid profile

The lipid profile required 35 μl blood that was drawn immediately after the killing. It was measured using the Cholestech LDX reflectance photometry system (Micro-Medical Instrumente, Königstein, Germany). It comprised total cholesterol, high-density lipoprotein cholesterol, triglycerides and glucose. Because of coagulation, reliable values could only be obtained for three out of six treatment groups.

Caffeine

The caffeine content in coffee samples from the mouse cages was measured by HPLC separation (phenylhexyl column, elution with methanol/water 50/50) and UV (272 nm) detection.

Morphology

Mice were killed by cervical dislocation at week 45. The pancreas was excised and fixed in 4% paraformaldehyde in 0.15 M phosphate-buffered saline, pH 7.3, and embedded for histological analysis. In addition, samples were fixed in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.3, post-fixed in 1% OsO_4, and finally embedded in Epon for electron microscopical analysis. For immunohistochemistry, serial paraffin sections were stained by the avidin-biotin-complex method as described.[29] Proliferating beta-cells were identified with the Ki67 antibody (C terminal, Acris, Herford, Germany) and apoptotic beta-cells with a terminal UTP nick end-labelling cell death detection kit (Roche, Mannheim, Germany) counterstained for insulin using immunofluorescence. For transmission electron microscopy (Zeiss EM 10C), 50-nm sections were cut by an ultramicrotome (Ultracut, Leica-Reichert-Jung, Germany), placed on nickel grids and contrast-stained with uranyl acetate and lead citrate.

Statistics

Statistical calculations were performed using SPSS (IBM, Ehningen, Germany) and the GraphPad Prism4 software (GraphPad, La Jolla, CA, USA). SPSS was used for two-way repeated-measures analysis of variance (ANOVA) with Huynh–Feldt correction for sphericity violation ('ANOVA', if not labeled otherwise). Where appropriate, one-way ANOVA or unpaired two-tailed Student's *t*-test were used. If not stated otherwise, 'significant' refers to the level of $P < 0.05$.

RESULTS

Coffee exposure

Coffee exposure of male C57BL/6NCrl mice started together with the onset of the HFD at week 10 and remained unchanged for the entire duration. Regular coffee (that is, coffee containing a natural content of caffeine) was offered as filtered brew of 20 g ground coffee per litre (mean caffeine concentration 327 ± 30 mg l^{-1}, $n = 29$) or 40 g l^{-1} (mean caffeine concentration 578 ± 50 mg l^{-1}, $n = 18$) for consumption *ad libitum*. Drinking water was offered *ad libitum* to the third group of mice on HFD. In addition, the effect of coffee consumption versus water consumption was observed in mice on normal maintenance diet (ND).

Effect on body weight

During exposure to coffee (see Figure 1) the body weight gain in both ND and HFD mice was significant as was the difference between ND and HFD (in both cases $P < 0.001$, ANOVA). In addition, coffee differentially affected body weight gain of ND and HFD mice ($P = 0.032$, ANOVA). During this time period, coffee significantly diminished the weight gain in ND mice ($P < 0.001$, ANOVA), but not HFD mice ($P = 0.196$, ANOVA). However, after week 32 the control HFD group did no longer gain weight, having reached the maximal weight of this mouse strain (Figure 1). Taking the ceiling effect of the control into account and limiting the time period of testing accordingly, the difference between control and coffee-consuming HFD mice was significant ($P = 0.034$, ANOVA). A significant dose-dependency ($P < 0.05$, one-way ANOVA) could be shown for the integrated values (area under the curve (AUC), not shown). HFD led to significant, but much less extensive, weight gain in female C57BL/6J mice.[30] As only marginal hyperglycaemia was attained, this model was not deemed suitable to investigate the diabetes-preventive effect of coffee consumption.

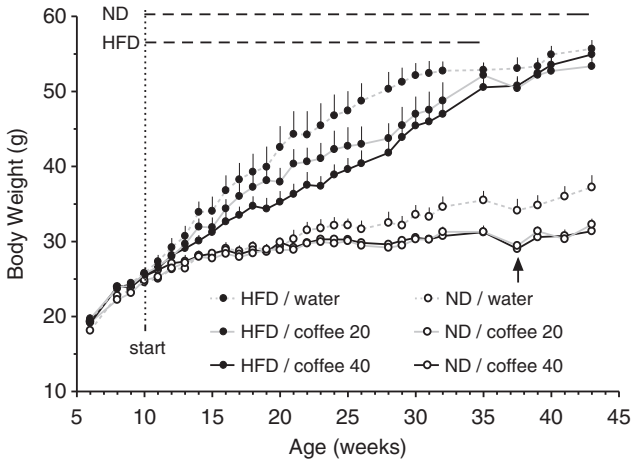

Figure 1. Effect of coffee consumption on weight gain in male C57BL/6NCrl mice. From week 10 on mice were either fed a HFD (closed circles) or a normal rodent diet (ND, open circles), from the same time point on fluid was offered as tap water (control = dashed grey line), coffee of low-to-moderate strength (20 g l^{-1} = solid grey line) or coffee of high strength (40 g l^{-1} = solid black line). The time periods selected for statistical testing of the effect of coffee consumption are given by the dashed lines (HFD, $P = 0.034$, ANOVA; ND, $P < 0.001$, ANOVA). The arrow indicates a weight measurement after overnight fasting. Data are means ± s.e.m. of five animals in each group (HFD control and HFD coffee 20: $n = 4$ after week 34).

Food and fluid consumption

At four time points, food consumption, fluid consumption, faecal weight and urine volume were measured during a 16-h overnight stay in a metabolic cage: baseline at week 8 and then after assigning to the treatment groups at weeks 18, 26 and 35 (Figure 2). At baseline (ND for all groups), the amount of consumed food was 3.17 ± 0.12 g ($n = 30$, Figure 2a). Switching to HFD reduced the amount to less than 2 g ($P < 0.001$, ANOVA) and it remained in that range for the entire observation period. There was no effect of time in the ND group ($P = 0.368$, ANOVA). Coffee consumption was significantly correlated with the food intake in the entire test population ($P = 0.045$, ANOVA). This correlation was only marginally significant in the ND group ($P = 0.066$, ANOVA) and absent in the HFD group ($P = 0.510$, ANOVA). Transforming the food consumption by weight into gross caloric intake (Figure 2b) showed that the control (water-consuming) mice on ND and HFD were not significantly different for the first 16 weeks. During that time both coffee-consuming groups on ND had a higher caloric intake than the corresponding groups on HFD.

The feeding efficiency was estimated based on the caloric intake measured at week 26 and the weight gain between week 25 and week 31, that is, a time when the weight gain of the water-consuming HFD mice already approached saturation. Assuming that 80% of the weight gain was due to fat, the rate of daily energy intake that was transformed into body mass was 5.9% for HFD mice on water, 6.9% for those on low strength and 5.4% for those on high-strength coffee. For the ND mice, the corresponding figures were 1.9% for control and less than 0.1% for both groups on coffee.

The fluid consumption (Figure 2c) was 5.28 ± 0.38 ml at baseline. In the HFD group, fluid consumption decreased strongly to ca. 2.6 ml and remained in that range for the entire period, whereas the consumption in the ND group was nonsignificantly increased to ca. 6.5 ml ($P < 0.001$, ANOVA and $P = 0.223$, ANOVA, respectively). In the HFD, but not the ND group, a significant dependence of the fluid consumption on the exposure to coffee was noted ($P = 0.007$, ANOVA and $P = 0.104$, ANOVA, respectively). In consequence, the caffeine intake of ND mice drinking high-strength coffee was about threefold higher than that of the corresponding HFD mice and about twofold higher for ND mice on low-strength coffee (Figure 2d).

The faecal weight was influenced by the type of food and by coffee exposure. It was 1.96 ± 0.06 g at baseline (ND) and decreased over time to 0.19 ± 0.01 g in the HFD control and to 1.24 ± 0.08 g the ND control group. Coffee-consuming mice (both strengths considered together) had a significantly higher faecal weight than the respective controls, namely 0.26 ± 0.2 g in the HFD and 2.25 ± 0.17 g in the ND group. Urine volume, in contrast, was not significantly affected. It was 0.91 ± 0.06 ml at baseline, and 1.38 ± 0.21 and 1.30 ± 0.17 ml for HFD and ND controls, respectively, and 0.96 ± 0.14 ml with coffee-consuming ml HFD mice and 0.89 ± 0.17 ml with coffee-consuming ND mice.

Levels of blood glucose, HbA1c and insulin

Routine blood glucose control was performed without a prior fasting period. This led to considerable data scattering (Figure 3a); however, in the population as a whole significant effects of time, diet and coffee could be verified ($P < 0.001$, $P = 0.001$ and $P = 0.034$, respectively, ANOVA). The time-dependent effect was mainly due to a decrease in blood glucose levels following week 21. This decrease was more easily visible in the HFD group because blood glucose levels were hyperglycaemic before week 21; however, LOWESS curves (locally weighted scatterplot smoothing) showed the same pattern of spontaneous decrease to exist also in the ND group (Figure 3b). In both, HFD and ND mice, this effect was highly significant ($P < 0.001$, ANOVA). Remarkably, at week 28 the mean values for both HFD and ND mice were significantly lower than the corresponding baseline values.

To test for coffee-dependent effects within the HFD and the ND groups, the mean blood glucose values for two periods of comparable length were calculated, from the start at week 10 to the first HbA1c measurement at week 26 and from week 26 until the last measurement at week 43 (Figure 4a). During the first, but not the second, period a significant difference between each subgroup in the HFD mice (control, low-strength coffee, high-strength coffee) and the corresponding subgroup in the ND mice occurred. However, no significant effect of coffee consumption within either HFD or ND mice could be detected (Figure 4a).

HbA1c was measured at weeks 26 and 41. The first measurement showed that coffee-consuming ND mice had significantly lower HbA1c values than the corresponding groups of HFD mice, whereas control ND mice were not different from control HFD mice (Figure 4b). At the second time point, no significant differences between the corresponding groups of HFD and ND mice were remaining (Figure 4b). The slight, but significant ($P = 0.036$, one-way ANOVA), correlation of the HbA1c value with coffee exposure within the HFD, but not the ND group, was no longer detectable at the second measurement.

The IPGTT at week 21 showed significantly higher fasting values for the HFD group than for the ND group (Figures 5a and b). The HFD control mice showed a delayed decrease in hyperglycaemia, whereas coffee-consuming HFD mice had maximal values in the same range at 15 min but significantly lower values at 30 min. At 120 min, a dose-dependent antihyperglycaemic effect ($P = 0.016$, one-way ANOVA) of coffee consumption was evident (Figure 5a). In the ND group, the pattern of all subgroups was closely similar, but in coffee-exposed mice, the decrease continued towards prestimulatory levels, leading to a significant difference at the last time point (Figure 5b). In the AUC areas, a clear inverse relation is visible between glycaemia and coffee consumption in the HFD but not the ND group (Figure 5e, left graph).

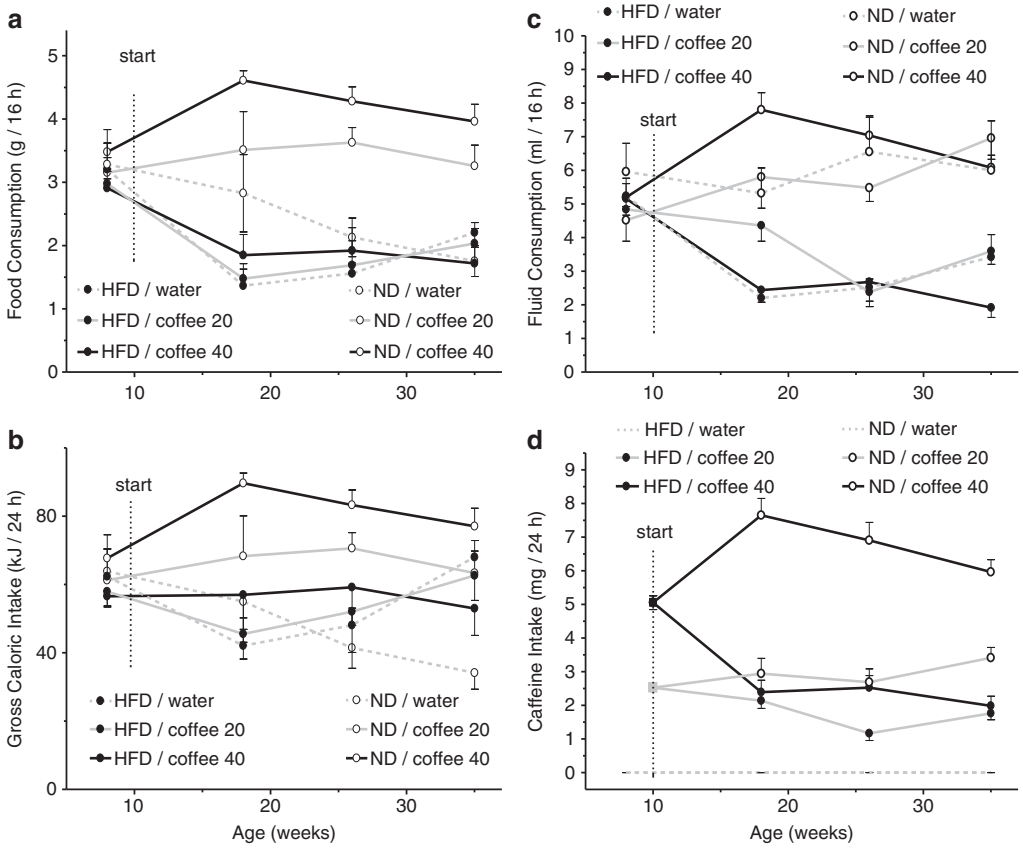

Figure 2. Food consumption (**a**, **b**) and fluid consumption (**c**, **d**) by HFD mice (closed circles) and ND mice (open circles). The treatment is indicated by the type of connecting line (water = dashed grey line, coffee 20 g l^{-1} = solid grey line, coffee 40 g l^{-1} = solid black line). (**a**) Coffee consumption was significantly correlated with the food intake in the entire test population ($P = 0.045$, ANOVA). (**b**) To make the data directly comparable between HFD and ND mice, the food consumption was corrected for the different caloric contents of the diets. (**c**) The fluid consumption of the HFD decreased significantly, whereas that of the ND mice remained unchanged ($P < 0.001$, ANOVA and $P = 0.223$, ANOVA, respectively). (**d**) Caffeine intake of the different treatment groups after correction for the measured levels of fluid consumption. Data are means ± s.e.m. of five animals in each group (HFD control and HFD coffee 20: $n = 4$ after week 34).

In the IPGTT of week 39, blood glucose excursions were lower than those of the same groups at week 21 (Figures 5c and d), even though fasting blood glucose values were not significantly different. In HFD mice, coffee consumption diminished the peak values at 15 and 30 min, resulting in a significantly reduced AUC for the first 30 min ($P < 0.05$, *t*-test for comparison AUC coffee versus AUC control), but not for the values at 60 and 120 min (Figure 5c). When glycaemia during both IPGTTs was analysed by two-way ANOVA, a significant effect of coffee consumption ($P = 0.021$) and of time ($P < 0.001$) was evident in the HFD mice (Figure 5e). In the ND mice, only the effect of time was significant ($P = 0.021$).

The change in response pattern of the HFD mice coincided with a large increase in the insulin levels, which were determined at $t = 60$ min of the IPGTTs (Figure 5f). Within the HFD group, high AUC values of glycaemia correlated with high insulin levels at week 21 but not at week 39. At the latter time point, insulin levels in the coffee-consuming ND mice tended to be lower than in the control ND mice (Figure 5f); however, this difference was only marginally significant ($P = 0.08$, *t*-test).

Serum lipids were determined in the treatment groups that showed significant differences in body weight at the end of the study (Table 1). Coffee-consuming ND mice and control ND mice had closely similar values for total cholesterol, triglycerides, high-density lipoprotein cholesterol and also blood glucose. The coffee-consuming HFD mice, in contrast, differed significantly from the corresponding ND mice in each single parameter.

Histological and ultrastructural examinations

Insulin-immunohistochemistry showed that in all treatment groups the islets were normally configured and had an intense staining of the beta-cells (Figure 6a). The size distribution showed no significant dependence on coffee consumption (Figure 6b); however, the mean size of the islets in the HFD groups was significantly larger than the size of the islets from ND-fed mice ($17\,082 \pm 1849\ \mu m^2$, $n = 123$ versus $9439 \pm 996\ \mu m^2$, $n = 105$, $P < 0.001$, *t*-test with Welch's correction). The occasional presence of lymphocytes in the vicinity of large islets from the HFD mice was not associated with an increased rate of apoptosis as indicated by the terminal UTP nick end-labelling reaction (Figure 6c) nor was there an increased rate of proliferation in the large islets at this time point as indicated by staining for Ki67 (Figure 6c). Ultrastructurally, the beta-cells in all treatment groups were well preserved with a normal granulation state. The only remarkable feature was a slight increase in the presence of secondary lysosomes. In particular, there were no signs of damage to the endoplasmic reticulum or the mitochondria in the HFD group (not shown).

DISCUSSION

The primary objective of this investigation was to reproduce in a mouse model of type 2 diabetes the epidemiological observation that chronic coffee consumption has a diabetes-preventive effect in humans. This is the necessary precondition for the experimental investigation of the currently unknown mechanism of action and

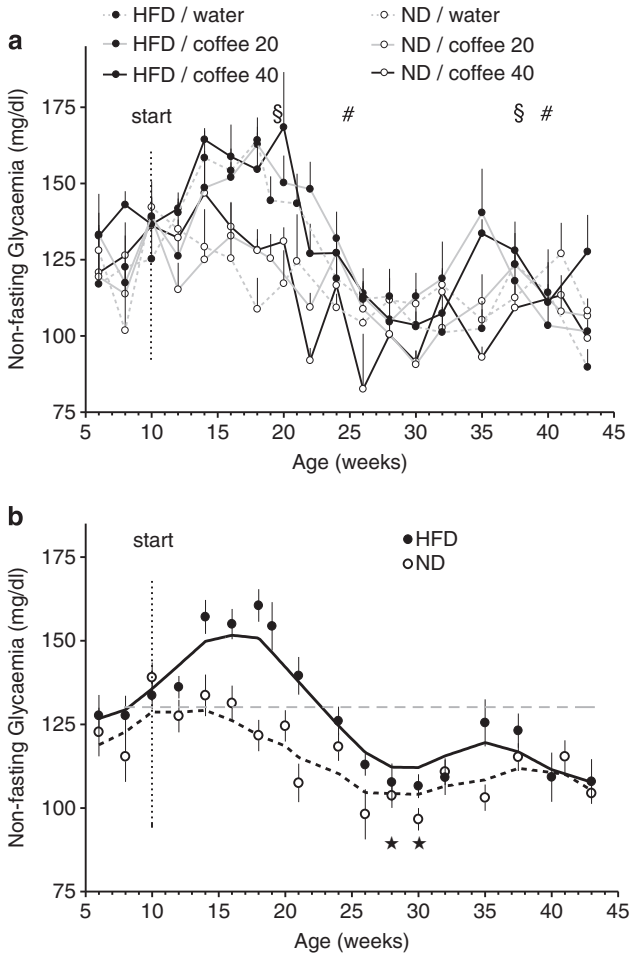

Figure 3. Development of non-fasting glycaemia in HFD mice (closed circles) and ND mice (open circles). (**a**) The data scattering obscures possible differences between the blood glucose values of HFD and ND mice consuming either high-strength coffee ($40\,g\,l^{-1}$ = solid black line) or low-strength coffee ($20\,g\,l^{-1}$ = solid grey line) or water (dashed grey line). The § signs denote the time points of the IPGTTs, the # signs those of the HbA1c measurements. Values are means ± s.e.m. of five animals in each group (HFD control and HFD coffee: $n = 4$ after week 34). (**b**) The mean glycaemia of HFD (closed circles) and ND (open circles) mice. The mean value of testing at weeks 8 and 10 was defined as the baseline (dashed grey line). The spontaneous decrease in glycaemia was significant with both groups ($P < 0.001$, ANOVA). Asterisks indicate time points where both HFD and ND values were significantly (*$P < 0.01$, *t*-test) lower than the baseline values. The connecting lines (solid = HFD, dotted = ND) are based on LOWESS curve fits. Values are means ± s.e.m. of 15 animals in each group (HFD: $n = 13$ after week 34).

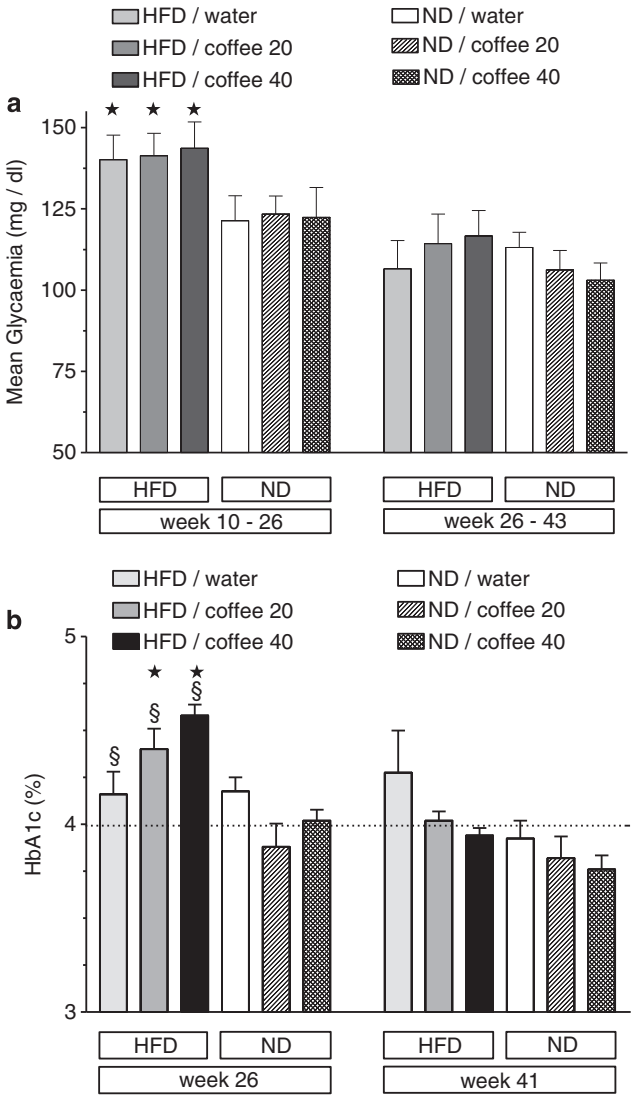

Figure 4. Comparison of the mean blood glucose values (**a**) and HbA1c levels (**b**) of mice on HFD (light grey = control, dark grey = coffee $20\,g\,l^{-1}$, black = coffee $40\,g\,l^{-1}$) or ND (open = control, hatched = coffee $20\,g\,l^{-1}$ or cross-hatched = $40\,g\,l^{-1}$) at weeks 26 and 41. (**a**) The mean blood glucose values were calculated for each treatment group for the 16-week period before the first HbA1c measurement and for the 17-week period before the second HbA1c measurement. During the first, but not the second, period non-fasting blood glucose was significantly higher in each HFD group than in the corresponding ND group (*$P < 0.05$, *t*-test). Values are means ± s.e.m. of nine measurements for the first and seven measurements for the second period. (**b**) HFD mice (solid bars) or ND mice (open, hatched or cross-hatched bars) showed only moderate differences in the levels of HbA1c. At week 26 the HbA1c values of the coffee-consuming ND mice were lower than those coffee-consuming HFD mice (*$P < 0.01$, *t*-test). At this time point the HbA1c of the HFD mice correlated ($^{§}P = 0.036$, one-way ANOVA) with coffee consumption (light grey = control, dark grey = $20\,g\,l^{-1}$, black = $40\,g\,l^{-1}$), but at week 41 this correlation was no longer existent. Values are means ± s.e.m. of five animals in each group (HFD control and HFD coffee 20: $n = 4$ at week 41).

should facilitate the identification of the relevant coffee constituents. However, it has to be kept in mind that lifespan, metabolism and nutrition differ from human living conditions and extrapolations have to be carried out with due care.

Body weight gain and fasting glucose values of water-drinking C57BL/6NCrl mice on HFD and ND corresponded to the values reported earlier[24,26] (see also website Charles River laboratories). The fluid consumption proved to be strongly dependent on the type of diet. As a general rule, ND mice consumed two- to three times more fluid and by consequence caffeine than HFD mice. The lower yield of water by carbohydrate oxidation than by lipid oxidation may have a role, and also, perhaps more importantly, the higher loss of water by ND mice due to higher faecal weight (difference 2 g versus HFD), which was not compensated by lower urine volume.

The caffeine content of filtered coffee is usually about 100 mg per cup or 60–80 mg per 100 ml.[31] The $20\,g\,l^{-1}$ coffee preparation, which resulted in a caffeine content of $327\,mg\,l^{-1}$, corresponds to a coffee of low-to-moderate strength, the $40\,g\,l^{-1}$ preparation ('high-strength coffee') to one of average strength in human consumption. If the caffeine intake of the mice

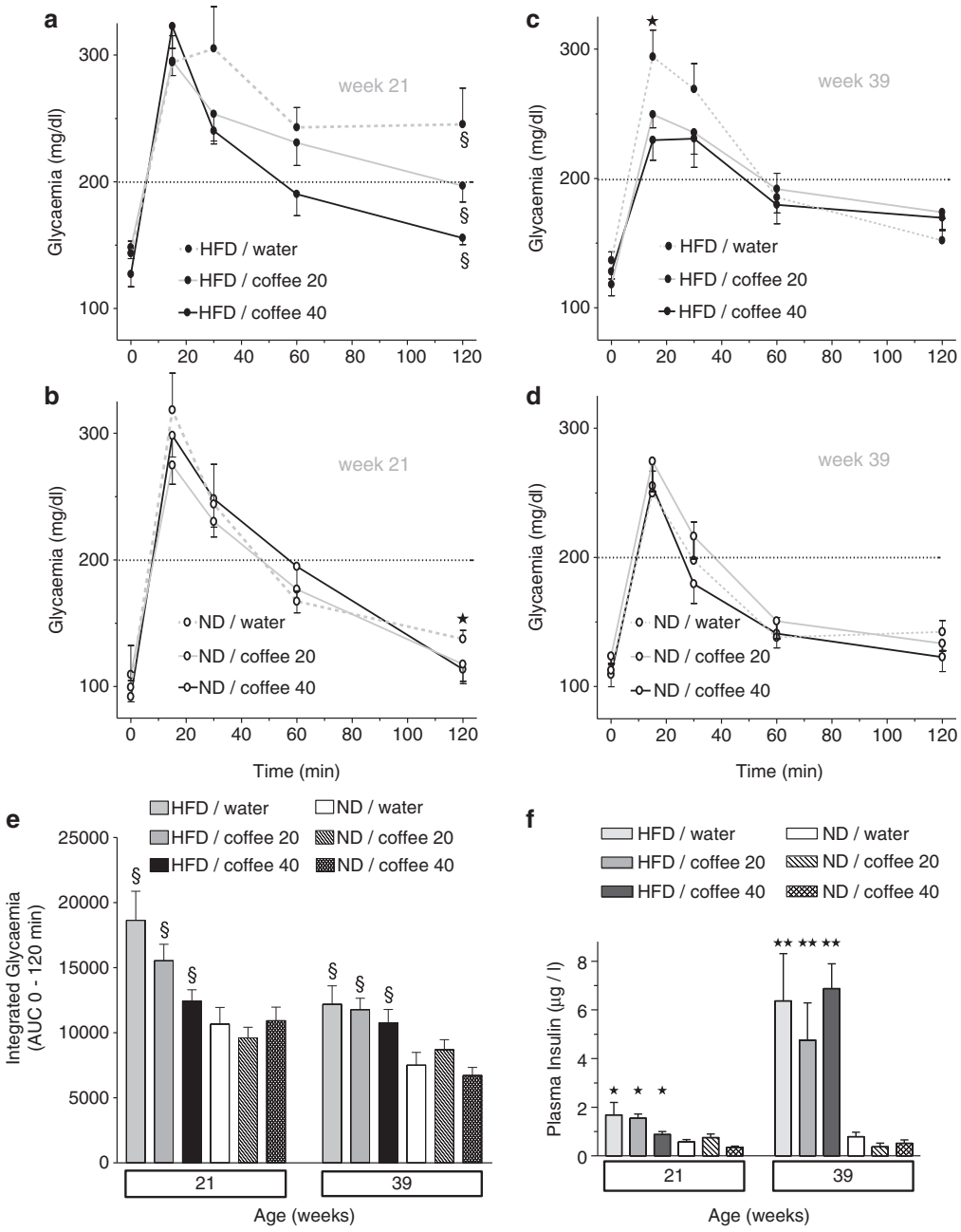

Figure 5. IPGTT during week 21 (**a**, **b**) and week 39 (**b**, **c**), the resulting integrated glycaemia (**e**) and pertaining insulin levels (**f**) at 60 min. (**a**) In the HFD group (closed circles), the coffee-exposed mice (20 g l^{-1} = solid grey line, 40 g l^{-1} = solid black line) showed a dose-dependent faster decrease in the elevated glucose levels ($^{\S}P = 0.016$, one-way ANOVA) than the control mice (dashed grey line). (**b**) In the ND group (open circles), a significant difference existed at 120 min between coffee-exposed mice and control mice (*$P < 0.05$, *t*-test). (**c**) At week 39 the coffee-exposed mice in the HFD group (solid grey and solid black lines) had lower peak glucose levels (*$P < 0.05$, *t*-test) than control mice (dotted grey line), but not lower values at 120 min. (**d**) In the ND group, the pattern was similar to that measured during week 21. (**e**) The integrated glycaemia shows that at both week 21 and week 39 there is a dose-dependent smaller AUC in HFD mice consuming coffee ($^{\$}P = 0.02$ for treatment, 0.001 for time, ANOVA), whereas only an effect of time was seen in ND mice. (**f**) In the IPGTT at week 21 all HFD groups had higher insulin levels than the corresponding ND groups (*$P < 0.05$, *t*-test). In the IPGTT at week 39 all insulin levels of HFD mice were significantly higher than in the first IPGTT (**$P < 0.05$, *t*-test). Values are means ± s.e.m. of five animals in each group (HFD control and HFD coffee 20: $n = 4$ at week 39).

(weighing between 25 and 54 g) is normalized with respect to human body weight (assuming 75 kg), it corresponds to a range of 20–200 cups per day in humans. However, if the higher metabolic rate of mice (63 kJ per day at baseline = 2.52 kJ g^{-1} per day) versus men (assuming 9250 kJ per day = 0.123 kJ g^{-1} per day) is considered instead, a correction factor of about 20 results. Therefore, the maximal intake of 7.5 mg caffeine per mouse and day would correspond to 150 mg per day in humans, or just 1.5 cups of regular coffee. As this value appears to be an underestimate as much as the former an overestimate, a correction for the different lifespan (ca. 40-fold) may be the most adequate procedure.

It was unexpected that the caloric intake of water-drinking mice on HFD and ND (final weight 54 and 36 g, respectively) was not

Table 1. Serum lipids and glucose at week 45

	Body weight (g)		*Total cholesterol (mg dl l^{-1})*		*HDL-cholesterol (mg dl l^{-1})*		*Triglycerides (mg dl l^{-1})*		*Blood glucose (mg dl l^{-1})*	
	Mean	*s.e.m.*	*Mean*	*s.e.m.*	*Mean*	*s.e.m.*	*Mean*	*s.e.m.*	*Mean*	*s.e.m.*
ND water	38.8	1.6	105.0	3.8	33.0	5.5	97.3	14.5	82.8	5.2
ND coffee (40 g l^{-1})	32.0	0.7[a]	106.4	4.4	28.8	4.2	97.0	12.2	81.4	8.0
HFD coffee (40 g l^{-1})	54.7	0.8[a]	126.3	3.2[a]	74.0	4.5[a]	130.0	9.0[b]	104.2	16.5[n.s]

Abbreviations: HDL, high-density lipoprotein; HFD, high-fat diet; ND, normal diet. Except for body weight, none of the parameters was significantly different between coffee-consuming and control ND mice. In contrast, HFD and ND mice consuming high-strength coffee differed. [a]$P<0.01$, [b]$P=0.06$ = marginally significant by each single lipid parameter. Values are means ± s.e.m. of four (ND-water) or five animals in each group.

significantly different during the first 26 weeks. A possible systematic error may consist of the measurement of food consumption only during the stay in the metabolic cage, a continuous monitoring of food consumption per treatment group might have been a useful complement. Literature data on the effect of HFD on caloric intake are conflicting, possibly because of a gender difference: a significantly higher caloric intake has been described for female C57Bl/6J mice on HFD[32] and a highly responsive subgroup of male HFD mice,[25] whereas no difference was seen in the total average of male HFD mice.[33,34]

However, the weight gain does not only result from increased energy intake, the present data clearly show an increase in feeding efficiency by HFD. Apart from a possible higher rate of nutrient absorption, the increase in feeding efficiency is probably due to a decrease in energy expenditure, that is, body temperature and/or locomotion. An inverse relation between weight and locomotor activity was found earlier within but not across mouse strains.[35] More recently, an acute onset of reduced locomotor activity upon HFD feeding was found and calculated to account for two-thirds of the weight gain.[34]

The maximal weight difference between coffee-consuming and control mice was 16% both in HFD and in ND mice. In view of the higher caloric intake of coffee-consuming versus control mice (both in HFD and ND groups), it is tempting to assume that the lower weight gain by coffee consumption (decreased feeding efficiency) results from an increased energy expenditure. In fact, an adenosine receptor-mediated increase in locomotion by caffeine was shown in rodents.[36,37]The increase in energy expenditure was also caused by coffee polyphenols without an increase, however, of locomotor activity.[38] Thus, two (or more) components in coffee may contribute to the inhibition of weight gain.

In marked contrast to our present observations, a recent study on the effects of coffee consumption on HFD-induced diabetes failed to detect an effect on body weight.[39] This discrepancy may be due to the lower fat content of the HFD in that study, the lower strength of the coffee preparation, the different substrain (C57BL/6J) and the shorter exposure time. The latter factor may be particularly important, as we observed the inhibition of weight gain even in ND mice, but this required 20 weeks to become significant.

Coffee consumption did not only diminish weight gain but also affected glucose homeostasis as evidenced by the IPGTT. The blood glucose values of the control HFD mice at weeks 21 corresponded well to values reported earlier for similar conditions.[28] At both time points (weeks 21 and 39), a dose-dependent antihyperglycaemic effect of chronic coffee consumption in HFD mice could be shown. However, the mechanisms appear to differ. During the first IPGTT, the peak values of the blood glucose were practically the same for coffee-consuming and control HFD mice, the large difference in the AUC being due to the faster decrease. As this was not associated with higher insulin levels, higher insulin sensitivity may be the underlying cause, possibly due to the lower weight of the coffee-consuming mice. An association between insulin sensitivity and the consumption of regular coffee was recently observed in non-diabetic adults.[14]

During the second IPGTT (week 39) in contrast the AUC of the first 30-min period was lower in coffee-consuming HFD mice, but the return to basal values was of the same velocity for all HFD mice. This may indicate an increased acute insulin response and is compatible with the threefold higher insulin levels in HFD mice at this point. In man, such a response was found to be associated with the consumption of decaffeinated coffee.[14] Interestingly, an improved glucose tolerance at week 39 versus week 21 was also found in ND mice, consistent with the spontaneous decrease in the non-fasting glycaemia. In fact, it has been reported early on that old and very old C57Bl/6J mice do not show a loss of beta-cell function but rather have moderately elevated plasma insulin levels and an enhanced biphasic secretion pattern *ex vivo*.[26]

The HbA1c levels were much lower than those in humans of comparable glycaemia but were within the range of previously reported values in mice.[40] The observation that the HbA1c level of HFD mice correlated with the coffee consumption at week 26 seems to be at odds with the hypothesis of diabetes-protective effect. However, the effect size was modest and did not persist at week 41. In addition, the calculation of the cumulative glycaemia during the weeks 10–26 did not confirm this relation while it confirmed the difference in HbA1c between HFD and ND mice. At week 41 neither HbA1c nor cumulative glycaemia indicated effects of HFD or coffee consumption. This concurs with the conclusion from the IPGTTs at week 39 that age as such does not impair the function of the endocrine pancreas. It also suggests that HFD does not lead to a permanent functional deterioration.

The strongly increased insulin secretion by HFD mice in the IPGTT at week 39 is consistent with the significantly enlarged islet area as compared with the islets from ND mice; however, Ki67 staining did not indicate an obviously increased proliferative activity.[41] In spite of the long exposure time and the massive obesity attained, there were no obvious signs of beta-cell damage or apoptosis in the islets from HFD mice, confirming that obesity is less directly linked to lipotoxic damage than often assumed.[42] Coffee consumption as such and the coffee-induced lower weight of ND mice had no effect on the morphology.

CONCLUSION

Consumption of regular coffee diminished weight gain in HFD mice and abolished weight gain in ND mice, probably due to a decrease in feeding efficiency. It is unclear whether the other major effect of coffee consumption, the improved glucose tolerance in HFD mice, is sufficiently explained by the diminished weight gain. Two factors contribute to the improvement. At the earlier time point, an increased insulin sensitivity has a role, later

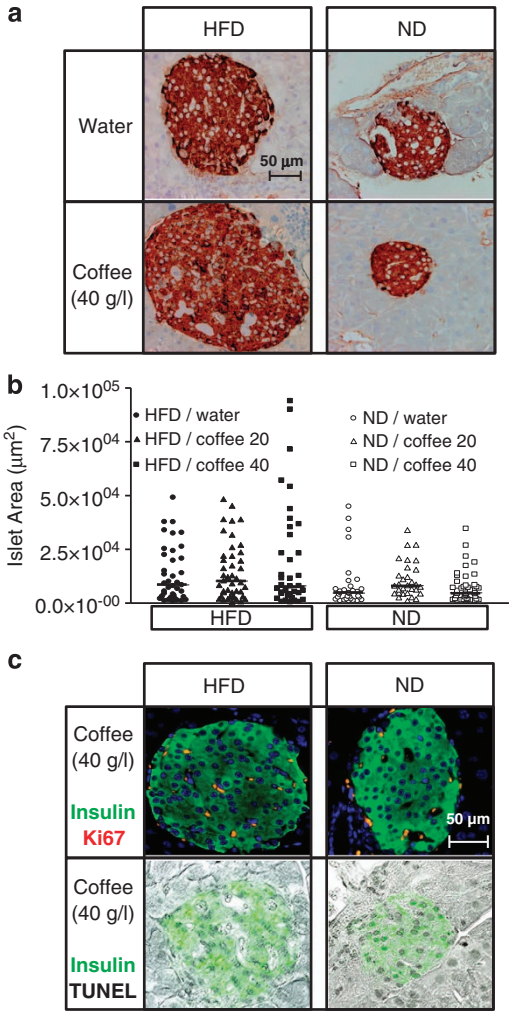

Figure 6. Morphology of the endocrine pancreas. (**a**) Insulin-immunohistochemistry of pancreatic islets after killing at week 45. Mice on HFD (left) and on ND (right) had normally configured islets with an intense insulin staining. (**b**) Scattergram and median value of the islet size distribution for each treatment group. Significant differences existed between HFD and ND islets but not between the different levels of coffee exposure. (**c**) Dual label immunofluorescence to assess beta-cell proliferation and apoptosis in islets from ND and HFD mice consuming high-strength coffee. Insulin-containing beta-cells are identified by the green fluorescence. The orange-red fluorescence (upper row) is linked to a Ki67 antibody detecting proliferating beta-cells, the greyish-black terminal UTP nick end-labelling (TUNEL) staining in the nuclei (lower row) indicates apoptotic beta-cells. The percentage of beta-cells showing Ki67- or TUNEL staining was not significantly affected by coffee consumption or HFD.

also an increased early insulin response. This situation is reminiscent of the current debate as to whether physical activity as such is beneficial to prevent type 2 diabetes beyond dietary restriction and independent of weight loss.[43–45] Overall, the observations suggest that the metabolic consequences of chronic coffee consumption in a mouse model of obesity and type 2 diabetes bear enough resemblance to the epidemiological findings in humans to encourage further experimental research into the relevant coffee components and their mechanism of action.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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