A CASE-CONTROL FIELD STUDY ON THE RELATIONSHIPS AMONG TYPE 2 DIABETES, SLEEPINESS, AND HABITUAL CAFFEINE INTAKE

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Abstract

Objectives: To examine the possible links between type 2 diabetes, daytime sleepiness, sleep quality and caffeine consumption.

Methods: In this case-control field study, comparing type 2 diabetic (n = 134) and non-type 2 diabetic (n = 230) participants, subjects completed detailed and validated questionnaires to assess demographic status, health, daytime sleepiness, sleep quality and timing, diurnal preference, mistimed circadian rhythms and habitual caffeine intake. All participants gave saliva under standardized conditions for *CYP1A2* genotyping and quantification of caffeine concentration. Hierarchical linear regression analyses examined whether type 2 diabetes status was associated with caffeine consumption.

Results: Type 2 diabetic participants reported greater daytime sleepiness ($p = 0.001$), a higher prevalence of sleep apnea ($p = 0.005$) and napping ($p = 0.008$), and greater habitual caffeine intake ($p < 0.001$), derived from the consumption of an extra cup of coffee each day. This finding was confirmed by higher saliva caffeine concentration at bedtime ($p = 0.01$). Multiple regression analyses revealed that type 2 diabetes status was associated with higher self-reported caffeine consumption ($p < 0.02$) and higher salivary caffeine ($p < 0.02$). Next to male sex, type 2 diabetes status was the strongest predictor of caffeine intake. Subjective sleep and circadian estimates were similar between case and control groups.

Conclusions: Type 2 diabetic patients may self-medicate with caffeine to alleviate daytime sleepiness. High caffeine intake could undermine efforts to control hyperglycemia and reflects a lifestyle factor that may be considered when promoting type 2 diabetes management.

Keywords

Vigilance, chronotype, coffee, *CYP1A2* genotype, HPLC, metabolism, sleep apnea

Introduction

Daytime sleepiness is a typical symptom of type 2 diabetes associated with hyperglycemia (West et al., 2006). Furthermore, daytime sleepiness is a symptom of obstructive sleep apnea, which is prevalent in persons with type 2 diabetes (Foster et al., 2009; West et al., 2006). The disordered breathing of sleep apnea causes repeated hypoxia and sleep fragmentation, and has been linked to increased insulin resistance and glucose intolerance, independent of the confounding effects of obesity (Ip et al., 2002; Punjabi et al., 2002). Elevated sleepiness may also be caused by disrupted sleep quality, sleep restriction, or misaligned circadian rhythms which themselves can impede glucose homeostasis via a decrease in insulin sensitivity (Buxton et al., 2010; Leproult et al., 2014; Tasali et al., 2008).

Caffeine intake reduces elevated sleepiness (Landolt et al., 2004). With this benefit in mind, caffeine is the most widely used psychoactive substance in the world (Fredholm et al., 1999). In the doses typically consumed, however, the stimulant increases the time it takes to fall asleep, shortens sleep duration and reduces sleep depth (Clark and Landolt, 2016; Landolt et al., 1995; Rétey et al., 2007). There may be a bidirectional link between daily caffeine consumption and sleep disturbance, leading to a vicious circle that promotes increased caffeine use: sleep problems lead to daytime sleepiness, and thus higher caffeine intake; yet, equally, caffeine consumption can lead to disturbed sleep and associated sleepiness (Roehrs and Roth, 2008).

There is a controversial discussion about caffeine's influence on glycemic control and type 2 diabetes risk (for review, see Palatini et al., 2015). Laboratory-based studies showed that doses of caffeine equivalent to 3-4 cups of coffee reduced glucose disposal by more than 20 % (Greer et al., 2001; Lane et al., 2004). Conversely, in epidemiology, high coffee consumption (6 cups/day) was related to a 33 % lower risk of type 2 diabetes (Ding et al., 2014). This paradox may reflect coffee's diverse composition, including the constituent compound chlorogenic acid that has beneficial effects on metabolism (Johnston et al., 2003). Susceptibility to the adverse effects of caffeine may also be

influenced by speed of caffeine metabolism by enzyme cytochrome P450-1A2 (CYP1A2), which has wide inter-individual variability in activity that is partly regulated by genetic polymorphism c.-163C>A (rs762551) of *CYP1A2* (Han et al., 2001; Sachse et al., 1999). This polymorphism causes inter-individual differences in the inducibility of CYP1A2 enzyme activity, for example by caffeine (Sachse et al., 1999). Slow metabolizers (i.e., C-allele carriers of rs762551) are more exposed to the adverse effects of caffeine, and thus potentially, are more at risk of developing type 2 diabetes following habitual high coffee consumption (Palatini et al., 2015).

The increasing burden of the type 2 diabetes pandemic (International Diabetes Federation, 2013) highlights the need to identify modifiable lifestyle factors that may help to prevent, treat and manage the progression of the disease. With this in mind, the present field study investigated the possible links between type 2 diabetes, sleep quality, and habitual caffeine consumption; as such relationships remain poorly understood. Based on the evidence presented above, it was hypothesized that type 2 diabetic patients would not only report greater daytime sleepiness, but also shorter sleep duration, poorer sleep quality, greater misalignment of circadian rhythms, and higher caffeine intake than non-type 2 diabetic controls. Moreover, there would be a higher proportion of slow CYP1A2 metabolisers in the type 2 diabetes group than in the controls.

Methods

Study outline

The study used a case-control approach that compared type 2 diabetic and non-type 2 diabetic participants. Subjects completed a series of questionnaires (the 'survey') to assess demographic status; health; sleep and chronotype; and habitual caffeine intake. Participants also gave two samples of saliva at bedtime: one sample was used to genotype participants for single nucleotide polymorphism rs762551 of the gene *CYP1A2*; the second sample was used to quantify the concentration of caffeine in saliva.

Survey data were checked thoroughly. Unrealistic responses were excluded from the analyses and counted as 'missing'; for example, if a subject was required to state a clock time, but instead reported a number greater than twenty four.

Due to genetic variation between populations of different ethnic origin, participants were screened for evidence of non-European descent and subsequently excluded. Subjects reporting type 1 diabetes, as opposed to type 2 diabetes, were also excluded, due to different risk factors associated with the two forms of the disease. To allow reliable assessment of typical sleep estimates across the groups, subjects reporting an extreme sleep schedule were excluded (sleep offset / wake-up time on work days > 12:00 / midday).

Subjects

The study was approved by the review board of the ethics committee at the Swiss Federal Institute of Technology (ETH) Zürich (ethics number: EK 2012-N-53). Experimental protocols were conducted according to the principles of the Declaration of Helsinki. The data were analyzed anonymously and individual results kept confidential. All participants gave written informed consent.

Participants were recruited via advertisements at hospitals, in magazines and at public seminars throughout German-speaking Switzerland. A total of 374 participants were recruited and their data collected. Ten participants were excluded based on: type 1 diabetes (n=2); non-European descent (n=7); and extreme sleep schedule (n=1). The remaining participants were split into the type 2 diabetic cases group (n = 134) and the non-type 2 diabetic controls group (n = 230). Of these participants, 11 cases and 27 controls chose not to provide saliva, only questionnaire data.

The type 2 diabetes status was determined by self-report. Specifically, an affirmative response to the question: "Over the past 12 months, have you suffered from type 2 diabetes?". This self-diagnosis was considered confirmed if the following criteria were met: *1)* The participant answered the

question: “If you suffer from type 2 diabetes, when did you receive your diagnosis?”; *2)* The participant reported a diabetes-appropriate treatment regime of oral medication and/or insulin.

Questionnaire assessment

The self-administered survey contained 6 questionnaires. Participants were instructed to complete the 20-minute survey in its entirety and to the best of their ability either online (2ask® survey software) or in paper form.

The first questionnaire gathered information regarding demographic and sociodemographic status, health and dietary behaviors, and chronic physical and mental health. Questions were based on those of a recent epidemiological study (Stamatakis et al., 2007).

Subjective sleep quality, sleep timing and chronotype assessment

The Pittsburgh Sleep Quality Index (PSQI) (Buysse et al., 1989) assessed subjective sleep quality and habitual sleep timing over the previous month. A higher score on the 0-21 scale reflects poorer subjective sleep quality (scores >5 indicate poor sleep). The Munich Chronotype Questionnaire (MCTQ) (Roenneberg et al., 2003) assessed sleep timing on work days and free days separately, and circadian variables. ‘Chronotype’ was determined as the midpoint between sleep onset and wake time on free days, corrected for ‘sleep debt’ accumulated during the work week (Roenneberg et al., 2012). A behavioral indicator of circadian misalignment (‘social jetlag’) was computed as the absolute difference between the midpoint of sleep on work days and free days (Roenneberg et al., 2012) (for further details and calculations, see supplementary information).

Daytime sleepiness and well-being assessment

The Epworth Sleepiness Scale (ESS) (Johns, 1991) measured participants' general degree of daytime sleepiness. A higher score on the 0-24 scale reflects greater subjective sleepiness (scores > 10 indicate excessive sleepiness). The World Health Organization Well-Being Index (WHO-5) (World Health Organization, 1998) assessed participants' general well-being and quality of life. A higher score on the 0-100 scale reflects greater well-being (scores < 50 indicate risk of depression).

Habitual caffeine intake assessment

This questionnaire was an extended version of the caffeine intake questionnaire of the sleep laboratory of the University of Zurich (Rétey et al., 2007). Participants were asked to report how frequently (per day or per week) they usually consumed a given range of caffeine-containing foods, drinks, medications and supplements. Table S1 (supplementary information) displays the estimated caffeine content (mg/serving) of each item in the questionnaire. These data were used to calculate participants' daily habitual caffeine intake.

Saliva sampling

Participants gave two samples of saliva at home and then posted them back to the laboratory in a pre-paid envelope. Beforehand, participants were posted a parcel containing detailed information, a checklist (to record time/date of sampling and caffeinated products consumed that day), and two saliva receptacles [*1)* Salivette® swab (Sarstedt, Nümbrecht, Germany); *2)* Oragene DNA kit (DNA Genotek Inc., Ottawa, Canada)]. Participants were instructed to give both saliva samples at bedtime, and without eating, drinking, chewing gum or smoking in the thirty minutes beforehand. Contact details of the research team were available in case participants needed assistance.

Genomic assessment with salivary DNA

Oragene receptacles were stored at room temperature until genomic DNA was extracted from saliva according to DNA Genotek Inc.'s instructions. Participants were genotyped for the functional rs762551 polymorphism of the *CYP1A2* gene, and labelled 'highly inducible' or 'less inducible' caffeine metabolizers (A/A genotypes = 'highly inducible'; A/C and C/C = 'less inducible'). All genetic analyses were replicated at least once for independent confirmation of the results. Experimental protocols are described in the supplementary information.

HPLC assessment of salivary caffeine

The saliva samples were delivered to the laboratory at room temperature. Upon receipt, the salivettes were stored immediately at -20°C. The stability of salivary caffeine concentrations over 14 days at room temperature has previously been confirmed (Perera et al., 2010). Salivary caffeine concentrations were quantified by high performance liquid chromatography (HPLC) coupled to a UV detector (Fuhr and Rost, 1994). Experimental protocols are described in the supplementary information.

The stability of salivary caffeine concentrations during long-term storage at -20°C was confirmed in a sub-sample (N=7). Saliva was analyzed at two time points, ten months apart. Statistical comparisons revealed that there was no significant difference between the caffeine concentrations at the two time points: 2.590 ± 1.573 (SD) *vs.* 2.523 ± 1.351 µg/ml ($p > 0.8$; paired-sample t-test).

Statistical analyses

Analyses were performed with Microsoft Excel 2010 (Microsoft Corp., Seattle, USA) and IBM SPSS Statistics 22 (IBM Corp., Armonk, USA). Mean values (± standard deviations) of raw data are reported and significance was set at $\alpha < 0.05$.

Continuous variables that were not normally distributed were transformed to approximate a normal distribution. The decision was based on visual inspection of the histogram and observation of the SPSS-derived skewness score. A skewness of less than -1 or greater than 1 reflects an unacceptable degree of skewness (Field, 2013). Table legends indicate successful transformation method.

Data from type 2 diabetes and non-type 2 diabetes groups were compared by Fisher's Exact Test (nominal data); independent samples t-test (normally distributed continuous data); or Mann-Whitney U test (not normally distributed continuous data that failed transformation attempts). Habitual caffeine intake of case and control groups was also analyzed by sex and smoking status (independent samples t-tests). HPLC-determined salivary concentrations of caffeine were compared to survey estimates of habitual caffeine intake using the Pearson's product-moment correlation. If data were missing for a variable, the smaller sample size for that variable was reported with the results.

Hierarchical multiple regression analysis was used to test the association between type 2 diabetes and habitual caffeine intake, after controlling for demographic, genetic, sleep, circadian, and work-structure variables. The outcome variable was self-reported habitual caffeine intake, transformed by the square root. The sixteen initial predictor variables were selected based on previous research (Cornelis et al., 2015; Penolazzi et al., 2012; Rodenburg et al., 2012). Predictors in the base model were nominal (binary) or continuous (normally distributed). Demographic variables were entered in the first step [age, sex, smoking, body mass index (BMI), well-being (WHO-5), sleep apnea, long-term medication, alcohol intake]. The *CYP1A2* genotype was entered in the second step. Sleep, circadian, and work-structure variables were added in the third step [subjective sleep quality (PSQI), napping, chronotype, night work, shift work, daytime sleepiness (ESS)]. In the final step, type 2 diabetes status was assessed. To achieve a parsimonious final model and avoid 'overfitting' (Field, 2013), statistically insignificant predictors were systematically tested to ascertain their contribution to the model (based on adjusted R^2). The final model was tested to ascertain that it met the statistical assumptions of multiple regression.

Results

Tables 1 and S2 (supplementary information) report and compare the type 2 diabetic cases and non-type 2 diabetic controls. Demographic and sociodemographic characteristics were similar between cases and controls; with the exception of male sex, BMI, relationship status and shift work. Within the type 2 diabetes group: 70.1% took oral anti-diabetic medication; 38.8% administered insulin; 43.6% were diagnosed 10 or more years ago.

The type 2 diabetic cases were in poorer physical and mental health than controls. For example, cases reported a greater incidence of high blood pressure, high cholesterol, and mental health disorder (Table 1). While reported physical activity and choice of water as most frequently consumed beverage did not differ between the groups, the type 2 diabetic participants reported lower alcohol consumption, less addition of sugar to hot drinks, and greater adherence to calorie-controlled or low-sugar diets. However, a greater proportion of type 2 diabetic cases were smokers.

Circadian and sleep estimates

Self-reported circadian and sleep estimates were similar between type 2 diabetic cases and controls (Table 1). However, sleep apnea and napping were more common in the type 2 diabetes group, and they reported greater daytime sleepiness.

Caffeine consumption

Estimated habitual caffeine consumption and salivary concentrations of caffeine are shown in Table 2. The type 2 diabetic cases reported consuming roughly 80 mg more caffeine each day than non-type 2 diabetic controls. Assessing men and women separately revealed that male type 2 diabetic patients consumed more caffeine than male controls (378.9 ± 212.1 *vs.* 277.5 ± 172.6 mg/day; $p <$

0.002); the difference was not significant in females (291.1 ± 183.4 *vs.* 251.0 ± 133.1 mg/day; p > 0.14) (Figure 1A). Separate assessment of non-smokers and smokers also revealed that non-smoking type 2 diabetic patients habitually consumed more caffeine each day than non-smoking controls (333.4 ± 203.3 *vs.* 257.1 ± 147.3 mg/day; p < 0.001) (Figure 1B). In smokers, the two groups did not differ (423.2 ± 208.4 *vs.* 315.5 ± 169.0 mg/day; p > 0.14). Greater reported caffeine intake in the type 2 diabetes group was corroborated by HPLC-assessment of saliva. At bedtime, salivary caffeine concentrations were higher in type 2 diabetic participants than in controls (2.69 ± 2.50 *vs.* 2.05 ± 1.94 µg/ml; p = 0.01). The self-reported caffeine intake was positively correlated with the salivary caffeine concentration (r = 0.317; p < 0.001) (Figure S1; supplementary information). The higher caffeine intake of type 2 diabetic cases stemmed from a greater intake of coffee, such that diabetic cases habitually consumed an extra cup of coffee each day (Table 2). Consumption of decaffeinated coffee was negligible. Figure S2 (supplementary information) illustrates dietary sources of caffeine for cases and controls.

[PLEASE INSERT FIGURE 1 ABOUT HERE]

CYP1A2 metaboliser status

The distribution of *CYP1A2 -163C>A* genotypes was similar in type 2 diabetes and non-type 2 diabetes groups (Table 1). The allele and genotype frequencies were comparable to published frequencies in older, non-patient control groups, of European descent (Popat et al., 2011).

Association between habitual caffeine intake and type 2 diabetes assessed by hierarchical multiple regression

The base model with 16 predictors (see Methods) predicted total habitual caffeine intake (ANOVA: $F_{16,181} = 3.7$; $p < 0.001$; adjusted $R^2 = 0.18$). Five predictors (age, sleep apnea, long-term medication, subjective sleep quality, night work) were subsequently excluded because they reduced the explanatory power of the model. The final parsimonious model predicted total habitual caffeine intake ($F_{11,189} = 5.7$; $p < 0.001$). The 11 predictors accounted for roughly 25 % of the variation in self-reported caffeine intake (Table 3, model 4)

Demographic variables explained 17.9 % of the variance in caffeine intake (model 1). The genetic predictor, *CYP1A2* genotype (model 2), did not significantly improve the explanatory power of the model ($p > 0.18$). By contrast, sleep, chronotype and work-structure variables (model 3) improved the predictive power of the model ($p = 0.04$). Daytime sleepiness did not help to predict caffeine intake ($p > 0.7$). The type 2 diabetes status was added in the final step (model 4) and was associated with total caffeine intake ($p < 0.02$). That is, when the effects of the other predictors were held constant, type 2 diabetes was associated with an increase in habitual caffeine intake. Furthermore, type 2 diabetes contributed to the model's explanation of the variance in habitual caffeine intake, such that compared to model 3, model 4 explained an additional 2.3 % of the variance in caffeine intake ($p < 0.02$). Overall, male sex was the strongest predictor of caffeine intake (Beta = -0.260), followed by type 2 diabetes status (Beta = 0.179) and then smoking status (Beta = 0.173). When salivary caffeine was substituted into the final model as the outcome variable, type 2 diabetes status was again associated with an increase in salivary caffeine ($p < 0.02$).

Discussion

The main finding of this field study is that habitual coffee consumption is an extra cup higher in type 2 diabetic patients compared to a control group. This result was confirmed by the analysis of salivary caffeine concentrations at bedtime. This finding is pertinent given the ongoing controversy over the relationship between coffee intake and risk of type 2 diabetes (Freedman et al., 2012; Palatini et al.,

2015). It supports our prediction that the diabetes group would consume more caffeine than controls.

Elevated sleepiness is a typical feature of type 2 diabetes (West et al., 2006). In line with this notion, the type 2 diabetic participants of this study reported greater daytime sleepiness than the control group, which was corroborated by the higher tendency to nap. The mean score on the Epworth Sleepiness Scale was similar to previous reports in type 2 diabetes samples (Foster et al., 2009; West et al., 2006).

Obstructive sleep apnea is also linked to sleepiness (West et al., 2006). Sleep apnea was more prevalent (12 %) in the present diabetes sample than in the controls (4 %), and the prevalence in the patient group was comparable to published reports (West et al., 2006). Type 2 diabetes and sleep apnea typically co-exist, and may have a bidirectional association by which each condition exacerbates the other (Moon et al., 2015). This field study lacked objective sleep measurements, and relied on questionnaires to assess the presence of sleep apnea. Consequently, it is impossible to differentiate the sleepiness derived from the poor nocturnal breathing in sleep apnea, from the sleepiness associated with diabetes. Both hyperglycemia and sleep apnea may be contributing to the increased sleepiness in the type 2 diabetes group.

Daytime sleepiness can also result from disturbed sleep and misaligned circadian rhythms which, in turn, can result in impaired glycemic control (Buxton et al., 2010; Leproult et al., 2014; Tasali et al., 2008). The diabetes group reported greater shift work than the control group, and shift work can lead to irregular sleep schedules and misalignment of circadian rhythms (Leproult et al., 2014). Nevertheless, self-reported sleep duration, subjective sleep quality, and a behavioral indicator of circadian misalignment ('social jetlag') were consistent between cases and controls. On average, participants slept for 7 hours and social jetlag was 41 minutes. These findings are in accordance with a large population-based, European sample, and are age-typical (Roenneberg et al., 2012). Similarly, both groups showed marginally poor sleep quality (PSQI > 5), but this is also a feature of normal

aging (Buysse et al., 1989; Bliwise et al., 2005). In conclusion, the congruence of the case and control groups' subjective sleep and circadian estimates implies that within the present sample, these behavioral factors may not play a key role in impeding glucose homeostasis. Equally, the similarity between groups of the distribution of the CYP1A2 enzyme inducibility suggests that this factor may not be a key determinant of reduced glycemic control in our type 2 diabetes sample.

It is plausible that the diabetic patients were self-medicating with caffeine to alleviate their sleepiness (Clark and Landolt, 2016; Roehrs and Roth, 2008). Indeed, statistical modelling revealed for the first time that type 2 diabetes status independently predicted higher habitual caffeine intake, both relying on self-report and measured caffeine levels in saliva. It has previously been shown that men and smokers tend to consume more caffeine than women and non-smokers (Penolazzi et al., 2012). These findings were reflected in the present model, with male sex and positive smoking status making significant contributions to the prediction of caffeine intake. Importantly, greater caffeine intake was reported by the male diabetic patients compared to the male control participants, suggesting that the higher caffeine use of the type 2 diabetes group does not reflect a bias of the higher male proportion in the patient group. Moreover, despite the higher prevalence of smoking in the type 2 diabetes group, there was no significant difference in caffeine consumption between case and control participants who currently smoke. The correlation coefficient between self-reported caffeine intake and salivary caffeine concentration in this study was similar to those reported in the literature (James et al., 1989) and validated the caffeine intake questionnaire.

After controlling for demographic, genetic, sleep, circadian and work structure variables already known to influence caffeine consumption (Cornelis et al., 2015; Penolazzi et al., 2012; Rodenburg et al., 2012), our model explained 25 % of the variation in caffeine intake. The negative contribution of daytime sleepiness to caffeine intake (Table 3) indicates that lower sleepiness was related to higher caffeine use. Although this contribution was weak and non-significant *per se*, this finding may suggest that across the entire cohort, the stimulatory effects of caffeine are experienced by participants. However, the occurrence of greater sleepiness and greater caffeine consumption in

type 2 diabetic patients implies that within this group, caffeine's stimulation may not be sufficient to outweigh the sleepiness associated with their condition.

On average, the Swiss population consumes 288 mg caffeine per day (Fredholm et al., 1999). Whereas the control group almost exactly matched the population average (261 mg), the present type 2 diabetic participants reported to consume substantially more (86 mg or 33%) at 347 mg caffeine per day. This amount is higher than the caffeine content in a regular cup of coffee (instant coffee = c. 57 mg) and most caffeine-containing, over-the-counter medications (Table S1). Importantly, objective caffeine quantification corroborated that salivary caffeine was 31% higher in the patient group. Such findings are clinically relevant because while a normal fasting blood glucose is 70-100 mg/dl, an elevated blood glucose, which signals diabetes, is a reading over 125 mg/ dl (American Diabetes Association, 2014); that is, an elevation of ≥ 25 %. The type 2 diabetes status was the strongest predictor of high caffeine intake, after male sex. Thus, above and beyond demographic, genetic, sleep, circadian and work structure variables, type 2 diabetes status contributed significantly to the variance in reported caffeine intake (Table 3).

Apart from the male sex bias and the relatively small number of female controls, which may have precluded a statistically significant difference between patient and control study participants in women, the study lacked objective measurements of glycemic control, sleep and circadian regulation. Given the observational nature of the investigation, the findings cannot address the direction of causality between variables, only highlight correlations and suggest plausible rationale for such relationships. Moreover, replication of this study, in a large, population-based sample, is recommended to assess the stability of the findings. Nonetheless, laboratory studies demonstrate that doses of caffeine (375 mg) such as those reported in the type 2 diabetes group impair glucose metabolism in resting humans through a transient increase in insulin resistance (Greer et al., 2001; Lane et al., 2004). Although the exact underlying mechanisms remain poorly understood, caffeine may interfere with extra- and intracellular processes of glucose signalling (Shearer and Graham, 2014).

Conclusion

The results of this study may indicate that type 2 diabetic patients consume large amounts of caffeine to alleviate daytime sleepiness. High caffeine intake could undermine efforts to treat hyperglycemia. On the other hand, the majority of the caffeine intake in this sample came from coffee and coffee contains bioactive compounds that may ameliorate the negative impact of caffeine on glycemic control (Johnston et al., 2003). In the present study, only 3 of 134 type 2 diabetic patients were advised to avoid caffeine. The American Diabetes Association and the European Association for the Study of Diabetes do not provide recommendations related to caffeine or coffee consumption for the management of type 2 diabetes (Inzucchi et al., 2012). Overall, caffeine and coffee consumption is potentially an important lifestyle factor that should be considered in the promotion of type 2 diabetes management.

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Declaration of conflicting interests

The authors declare no potential conflict of interest, financial or otherwise.

Contribution statement

HPL proposed the trial concept and design and secured the funding. EU, GAS, WL and HPL contributed to recruitment of patients and controls, data collection, data analyses and interpretation, and discussion of the results. AJ (caffeine quantification in saliva), SCH and WB (genotyping) contributed analytic tools. EU and HPL wrote the manuscript. All authors contributed to the reviewing and editing of the manuscript, and approved the final version.

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Figure 1. Total habitual caffeine intake of type 2 diabetes and non-type 2 diabetes groups split by sex (A) and smoking status (B). Boxplots represent self-reported total habitual caffeine intake (box: 25th percentile, median and 75th percentile; whiskers: 10th to 90th percentiles; dots: individual data points outside of the whisker range). The estimates of caffeine consumption were based on the caffeine content reported by manufacturers of Swiss products or the website 'Caffeine Informer' (Table S1; supplementary information). Statistics compared type 2 diabetes (male: n = 85; female: n = 49; non-smoking: n = 114; smoking: n = 20) and non-type 2 diabetes (male: n = 84; female: n = 146; non-smoking: n = 216; smoking: n = 14) groups with independent samples t-test on square-root data (2-tailed).

Table 1. Characteristics of type 2 diabetes and non-type 2 diabetes groups.

Variable	Type 2 Diabetes (n=134)	Non-Type 2 Diabetes (n=230)	*P*-value
DEMOGRAPHIC DATA			
Age (years)	64.1 (±9.7)	63.8 (±9.9)	0.738
Male sex (%)	63.4	36.5	**<0.001**
Body mass index (BMI; kg/m^2)	28.8 (±5.5)	23.9 (±3.5)	**<0.001**
Night work (% yes)[a]	8.3	3.5	**(0.054)**
Shift work (% yes)[b]	6.0	1.8	**0.036**
HEALTH BEHAVIOURS			
Smoking (% yes)	14.9	6.1	**0.008**
Alcohol intake (% yes)[c]	31.3	43.5	**0.026**
Physical activity (% yes)[d]	50.0	56.5	0.233
Avoid caffeine to avoid sleep disruption (% yes)[e]	46.0	61.0	**0.009**
DIETARY BEHAVIOURS			
Beverage consumed when thirsty (% water)[f]	52.2	43.9	0.129
Most frequently consumed beverage (% water)[f]	51.5	52.2	0.914
Sugar in hot drinks (% yes)[g]	6.5	20.5	**<0.001**
Artificial sweeteners in hot drinks (% yes)[h]	26.8	10.4	**<0.001**
Dietary regime (%)			
o None	66.4	80.0	**0.006**
o Calorie-controlled or low sugar	28.4	8.3	**<0.001**
o Vegetarian, vegan or 'other'	5.2	11.7	**0.041**
Nutritional, herbal, vitamin supplements (% yes)[i]	23.5	35.4	**0.024**
CARDIOVASCULAR HEALTH			
High blood pressure (% yes)	47.8	17.4	**<0.001**
High cholesterol (% yes)	35.8	14.3	**<0.001**
Heart disorder / problem (% yes)	10.4	6.1	0.154
MENTAL HEALTH			

Mental health disorder (% yes)		20.1	12.2	**0.048**
Depression (% yes)		15.7	8.7	**(0.058)**
Well-being / Quality of life (WHO-5)		49.3 (±25.5)	53.2 (±26.0)	0.166
MEDICAL INTERVENTION				
Advised to avoid to caffeine (% yes)[p]		2.2	0.9	N/A
Long-term medication (% yes)		97.8	54.3	**<0.001**
Oral medication for diabetes (% yes)		70.1	-	N/A
Insulin injections for diabetes (% yes)		38.8	-	N/A
CIRCADIAN ESTIMATES (MCTQ)				
Chronotype metric[k]		03:18 (±01:02)	03:22 (±00:52)	0.592
Social jetlag (min)[l]		40.8 (±43.4)	41.4 (±36.6)	0.483
Average sleep duration (h)[m]		7.15 (±1.14)	7.15 (±1.02)	0.989
ESTIMATES OF SLEEP QUALITY				
Habitual sleep duration (PSQI, h)[n]		6.93 (±1.30)	6.90 (±1.09)	0.790
Habitual time in bed (PSQI, h)[n]		8.14 (±1.33)	8.14 (±1.14)	0.955
Sleep efficiency (PSQI, %)[n]		85.9 (±11.9)	85.3 (±11.0)	0.568
Sleep latency (PSQI, min)[o]		19.3 (±19.1)	20.2 (±18.4)	0.283
Subjective sleep quality (PSQI)[p]		6.2 (±3.5)	5.6 (±3.5)	0.114
Trouble sleeping (% yes)[q]		50.4	46.3	0.513
Insomnia (% yes)		25.4	28.7	0.544
Sleep apnoea (% yes)		11.9	3.9	**0.005**
Napping (% yes)[r]		33.1	20.0	**0.008**
Daytime sleepiness (ESS)[s]		8.4 (±4.2)	6.8 (±3.5)	**0.001**
***CYP1A2* GENETIC CHARACTERISTICS**[t]				
Allele frequency (%)	A	74.8	71.0	0.317
	C	25.2	29.0	
Genotype frequency (%)	A/A	59.7	50.7	0.180
	C/A	30.3	40.5	
	C/C	10.1	8.8	

Enzyme inducibility (%)[u]	High	59.7	50.7	0.133
	Less	40.3	49.3	

Abbreviations: T2D, type 2 diabetes; Non-T2D, non-type 2 diabetes; N/A, not applicable; MCTQ, Munich ChronoType Questionnaire; PSQI, Pittsburgh Sleep Quality Index; ESS, Epworth Sleepiness Scale; SNP, single nucleotide polymorphism. Data for continuous variables are means (± standard deviation) of raw data. *P*-values (2-tailed) were calculated using independent samples t-tests, comparing T2D and Non-T2D groups. . Data for categorical variables are %. *P*-values (exact; 2-tailed) were calculated using Fisher's exact test.

Raw data of age and BMI were log10-transformed to achieve a normal distribution. [a] T2D: n=133; NonT2D: n=230; [b] T2D: n=133; NonT2D: n=228; [c] Consume 3 or more alcoholic drinks per week; [d] Physical activity raising breathing and heart rate at least 3 times per week; [e] T2D: n=124; NonT2D: n=210; [f] Options: water, milk, cola/energy drink (caffeinated), diet cola/energy drink (caffeinated), soda (caffeine free), diet soda (caffeine free), fruit juice, herbal tea, coffee, black tea, decaffeinated coffee, decaffeinated tea, beer, wine, spirit; [g] T2D: n=123; NonT2D: n=210; [h] T2D: n=123; NonT2D: n=211; [i] T2D: n=132; NonT2D: n=226; [n] T2D (n=133); [j] T2D: n=134; NonT2D: n=228; [k] T2D: n=99; NonT2D: n=132; [l] T2D: n=85; NonT2D: n=95. Raw data transformation: Reciprocal; [m] T2D: n=99; NonT2D: n=135; [n] T2D: n=124; NonT2D: n=226; [o] T2D: n=132; NonT2D: n=229. Raw data transformation: Log10; [p] T2D: n=125; NonT2D: n=222. Raw data transformation: Square root; [q] T2D (n=133); NonT2D (n=229); [r] Habitually nap during the day at least 3 times per week. T2D: n=133; NonT2D: n=230; [s] T2D: n=125; NonT2D: n=209. Raw data transformation: Square root; [t] SNP rs762551.T2D: n=119; NonT2D: n=205; [u] Highly inducible = genotype A/A; Less inducible = genotypes A/C and C/C.

Table 2. Caffeine consumption based on self-reported caffeine intake (survey) and objective salivary caffeine concentrations (HPLC-derived).

Caffeine Consumption	**Type 2 Diabetes (n=134)**	**Non-Type 2 Diabetes (n=230)**	***P*-value**
Total habitual caffeine intake (mg/day)[a]	346.8 (±205.8)	260.7 (±148.9)	<0.001
o Caffeine from coffee (mg/day)[ab]	292.9 (±206.1)	211.1 (±151.7)	<0.001
o Total cups of coffee per day[cd]	3.47 (±2.46)	2.53 (±1.69)	<0.001
o Cups of caffeinated coffee per day[c]	3.43 (±2.47)	2.47 (±1.69)	<0.001
o Cups of decaffeinated coffee per day	0.04 (±0.18)	0.06 (±0.21)	0.243
Salivary caffeine concentration (µg/ml)[ae]	2.69 (±2.50)	2.05 (±1.94)	0.010

Abbreviations: T2D, type 2 diabetes; Non-T2D, non-type 2 diabetes. Data are means (± standard deviation) of raw data. *P*-values (2-tailed) were calculated using independent samples t-tests, comparing T2D and Non-T2D groups. Raw data was transformed to achieve a normal distribution (method of transformation noted in legend). The exception was 'decaffeinated coffee cups/day' data which failed transformation attempts; here, the *P*-values (exact 2-tailed) reflect Mann-Whitney U test.

[a] Raw data transformation: Square root; [b] Includes caffeine from decaffeinated coffee (4.5 mg/cup); [c] Raw data transformation: Log10; [d] Includes caffeinated and decaffeinated coffee; [e] T2D (n=123); Non-T2D (n=203).

Table 3. Hierarchical regression analysis to predict total habitual caffeine intake (N=200).

CATEGORY	VARIABLE	MODEL 1			MODEL 2			MODEL 3			MODEL 4		
		B	*Beta*	*P*	*B*	*Beta*	*P*	*B*	*Beta*	*P*	*B*	*Beta*	*P*
DEMOGRAPHIC	Sex *(Ref: Male)*	-2.605	-0.236	**0.001**	-2.654	-0.240	**0.001**	-3.123	-0.283	**<0.001**	-2.870	-0.260	**<0.001**
	Smoking *(Ref: No)*	3.084	0.167	**0.012**	3.167	0.172	**0.010**	3.367	0.183	**0.006**	3.189	0.173	**0.008**
	Body Mass Index	17.098	0.251	**<0.001**	16.936	0.249	**0.001**	14.916	0.219	**0.003**	9.455	0.139	0.079
	Well-Being	-0.030	-0.131	**0.049**	-0.029	-0.131	**0.050**	-0.026	-0.114	0.087	-0.022	-0.100	0.129
	Alcohol Intake *(Ref: No)*	0.911	0.080	0.243	0.963	0.084	0.217	0.895	0.078	0.245	0.977	0.085	0.199
GENETIC	*CYP1A2* Inducibility *(Ref: High)*				-0.956	-0.086	0.189	-0.816	-0.073	0.259	-0.661	-0.059	0.356
SLEEP, CIRCADIAN & WORK STRUCTURE	Napping *(Ref: No)*							-1.592	-0.109	0.105	-1.614	-0.111	0.096
	Chronotype							0.529	0.093	0.153	0.563	0.099	0.124
	Shift Work *(Ref: No)*							3.855	0.137	**0.044**	3.669	0.130	**0.053**
	Daytime Sleepiness							-0.200	-0.023	0.732	-0.274	-0.031	0.634
BLOOD GLUCOSE REGULATION	Type 2 Diabetes *(Ref: Non-Type 2 Diabetes)*										1.994	0.179	**0.017**
	R^2	*0.179*			*0.186*			*0.228*			*0.251*		

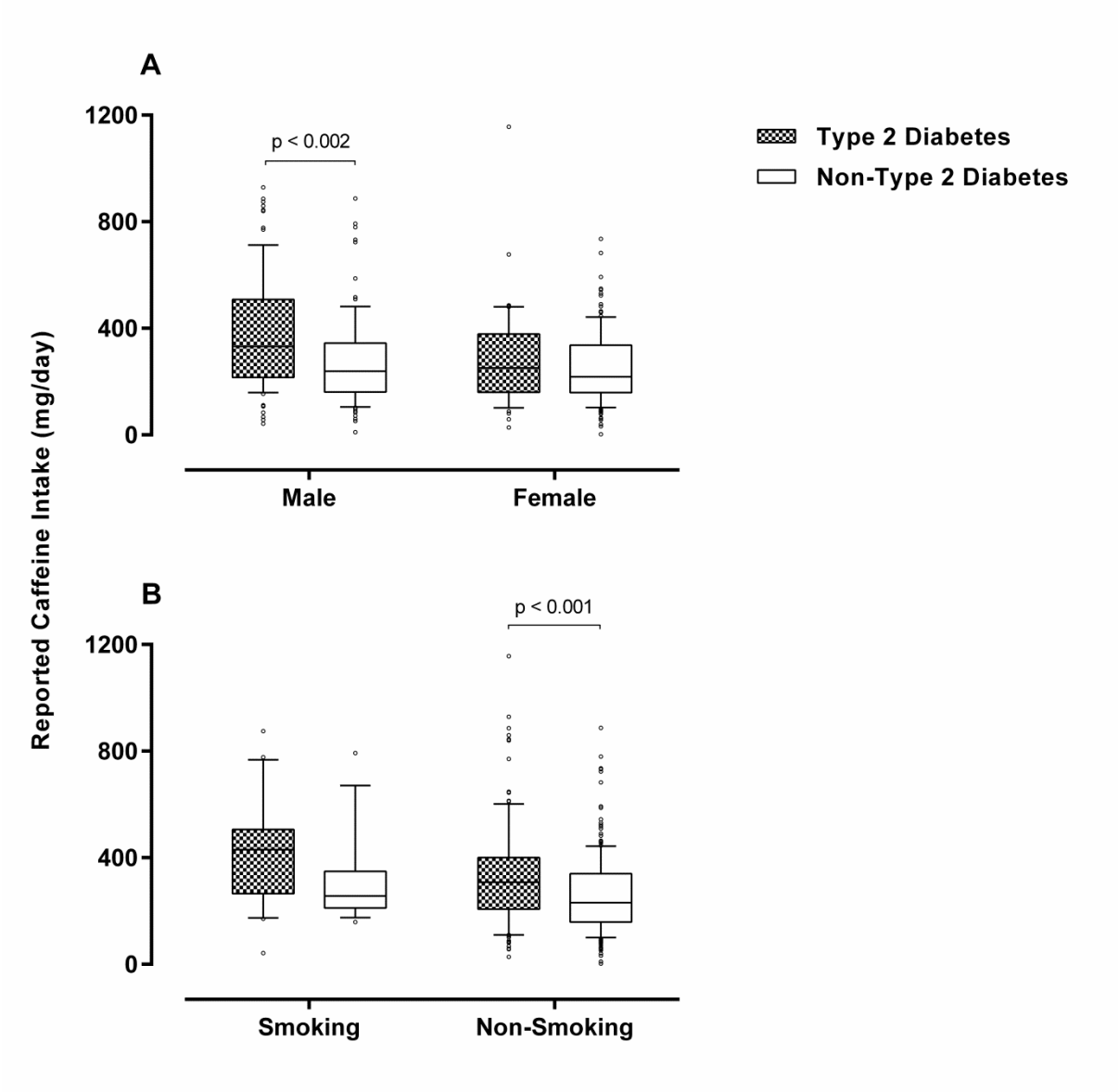
	Adjusted R^2	*0.158*			*0.161*			*0.187*			*0.207*		
	Change in R^2				*0.007*		*0.189*	*0.041*		*0.041*	*0.023*		*0.017*

Abbreviations: B = unstandardized coefficient; Beta = standardized coefficient; *P* = *P*-value; Ref = reference.

Table represents final model of 11 variables predicting survey-derived total habitual caffeine intake. Continuous variables were either normally-distributed raw data [well-being (WHO-5), chronotype metric] or raw data transformed to achieve a normal distribution [total habitual caffeine intake (square root), body mass index (Log10), daytime sleepiness (ESS; square root)]. Categorical variables were binary.

The base model contained 16 predictors: Demographic variables: age, sex, smoking, body mass index, well-being, sleep apnea, long-term medication, alcohol intake. Genetic variable: CYP1A2 extent of inducibility. Sleep, Chronotype and Work Structure variables: subjective sleep quality, napping, chronotype, night work, shift work, daytime sleepiness. Blood Glucose Regulation: type 2 diabetes.

Figure 1


A
1200
800
400
0
p < 0.002
Male
Female
Type 2 Diabetes
Non-Type 2 Diabetes
Reported Caffeine Intake (mg/day)
B
p < 0.001
Smoking
Non-Smoking

A CASE-CONTROL FIELD STUDY ON THE RELATIONSHIPS AMONG TYPE 2 DIABETES, SLEEPINESS, AND HABITUAL CAFFEINE INTAKE

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Supplementary Information

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Table S1: Caffeine content of products available for consumption in German-speaking Switzerland

Caffeine product	Size of serving (ml)	Total caffeine per serving (mg)	Information source (website)
COFFEE			
Expresso-based coffee. Single shot (e.g. expresso, latte, cappuccino, mocha.)	44	77	Caffeine Informer [a]
Instant coffee	240	57	Caffeine Informer
Brewed/filter coffee	240	107.5	Caffeine Informer
Decaffeinated coffee	240	4.5	Caffeine Informer
COLD COFFEE ('Emmi cafe latte')			
Cappuccino	230	80	Manufacturer
Caramel	230	60	Manufacturer
Expresso	230	120	Manufacturer
Light	230	80	Manufacturer
Macchiato	230	80	Manufacturer
Tahiti	230	60	Manufacturer
Zero	230	110	Manufacturer
TEA			
Brewed/loose-leaf black tea	240	48	Caffeine Informer
Green tea/white tea	240	25	Caffeine Informer
ENERGY DRINKS			
Redbull	355	114	Manufacturer
Redbull	250	80	Manufacturer
Redbull (sugar free)	250	80	Manufacturer
Migros own brand	250	80	Manufacturer
Migros own brand (sugar free)	250	80	Manufacturer
OK energy drink	355	114	Manufacturer
OK energy drink (light)	250	80	Manufacturer
Coop own brand	250	75	Manufacturer
Coop own brain (sugar free)	250	75	Manufacturer
Monster Energy	500	160	Manufacturer
Rockstar Energy Drink	500	160	Manufacturer
Lucozade	380	46	Manufacturer

Relentless Energy drink	500	160	Manufacturer
SOFT DRINKS			
Coca cola, Pepsi, flavoured cola, shop-branded cola	330	38	Manufacturer
Coca cola, Pepsi, flavoured cola, shop-branded cola	500	58	Manufacturer
Diet coca cola, diet Pepsi, Coke Zero, Pepsi Max, shop-branded, diet flavoured cola	330	38	Manufacturer
Diet coca cola, diet Pepsi, Coke Zero, Pepsi Max, shop-branded, diet flavoured cola	500	58	Manufacturer
Caffeine-free Diet Coca Cola	330 or 500	0	Manufacturer
Dr Pepper	330	38	Manufacturer
Dr Pepper	500	58	Manufacturer
Iced tea (e.g. Lipton, Nestea)	330	22	Manufacturer
Iced tea (e.g. Lipton, Nestea)	500	33	Manufacturer
Iced tea (light/zero)	330	22	Manufacturer
Iced tea (light/zero)	500	33	Manufacturer
DRINKING CHOCOLATE			
Hot chocolate (e.g. Suchard Express, Caotina)	240	7	Manufacturer
Cold chocolate (e.g. Nesquik, Micao, Comella)	240	7	Manufacturer
SOLID CHOCOLATE			
Milk	25g	6.25	Manufacturer
Dark	25g	17	Manufacturer
ICE CREAM			
Coffee-flavoured ice cream (e.g. Haagen-Dazs, Ben & Jerry)	240	59	Manufacturer
CAFFEINE PILLS / SUPPLEMENTS			
Caffeine pills (e.g. ProPlus)	2 tablets	100	Manufacturer
Supplements for general fatigue / lack of well-being (e.g. Tonikum D Flussig)	10	0.9	Manufacturer
MEDICATION			
Cold and Flu (e.g. Rhinitin retard, Rhin-X)	1 capsule	25	Manufacturer

Painkillers (e.g. Contra-Schmerz, Migrane-Kranit, Panadol Extra)	500 mg	65	Manufacturer
Anti-nausea / motion sickness (e.g. Itinerol B6)	2 capsules	40	Manufacturer

Note: If there were several brands available for a given caffeine-containing product (e.g. Lipton and Nestea Iced Tea), the estimated caffeine content was averaged across the brands.

[a] Caffeine informer website: http://www.caffeineinformer.com/the-caffeine-database

Table S2. Type 2 diabetes duration and sociodemographic characteristics.

Variable	Type 2 Diabetes (n=134)	Non-Type 2 Diabetes (n=230)	*P*-value
TYPE 2 DIABETES DURATION			
o < 1 year (%)	8.3	-	N/A
o 1-3 years (%)	15.0	-	N/A
o 4-6 years (%)	20.3	-	N/A
o 7-9 years (%)	12.8	-	N/A
o ≥ 10 years (%)	43.6	-	N/A
SOCIODEMOGRAPHIC DATA			
Relationship status (%)			0.159
o Never married	11.9	17.0	0.226
o Married	67.9	55.7	**0.026**
o Separated or divorced	17.2	23.0	0.229
o Widowed	3.0	4.3	0.585
Home ownership (% owner)	51.5	55.7	0.448
Household density (no. persons/no. rooms)[a]	0.4 (±0.2)	0.5 (±0.2)	0.640
Education (continued after high school; % yes)	98.5	97.0	0.495
Work (% yes)[b]	50.0	49.3	0.913
Work (hours per week)[c]	16.7 (±20.2)	15.6 (±19.1)	0.807

Abbreviations: T2D, type 2 diabetes; Non-T2D, non-type 2 diabetes; N/A, not applicable. Data for continuous variables are means (± standard deviation) of raw data. *P*-values (2-tailed) were calculated using independent samples t-tests, comparing T2D and Non-T2D groups, on raw data. If raw data was abnormally distributed, the data was transformed to achieve a normal distribution before the t-test was applied. Data for categorical variables are %. *P*-values (exact; 2-tailed) were calculated using Fisher's exact test.

[a] Raw data transformation: Reciprocal; [b] % working >0 hours per week; T2D: n=132; NonT2D: n=223; [c] T2D: n=133; NonT2D: n=223. Raw data transformation: Log10.

Experimental Procedures

Calculations on data derived from Pittsburgh Sleep Quality Index (PSQI)

Habitual time in bed = typical bedtime vs. typical get-up time

Habitual sleep duration = habitual time in bed – typical sleep latency*

**sleep latency = time taken to fall asleep*

Sleep efficiency = (habitual sleep duration / habitual time in bed) x 100

Calculations on data derived from Munich Chronotype Questionnaire (MCTQ)

Note: workers were assumed to have 5 work days and 2 free days each week; non-workers and retired participants were assumed to have 7 free days each week. However, if a non-worker or retired person reported separate work and free day timings, then these timings were used in the analysis.

Sleep duration = sleep onset (bedtime + sleep latency) vs. wake time

Average sleep duration = [(work day sleep duration x 5) + (free day sleep duration x 2) / 7]

Chronotype metric = midpoint of sleep on free days – (0.5 x sleep debt*)

**sleep debt = free day sleep duration – average sleep duration*

Social jetlag = midpoint of sleep on work days vs. midpoint of sleep on free days

Genomic assessment with salivary DNA

Genomic DNA was extracted from saliva according to DNA Genotek's instructions. Participants were genotyped for the cytochrome P450-1A2 gene (*CYP1A2*. -163C>A. SNP ID: rs762551. Assay ID: C_8881221_40) with TaqMan SNP Genotyping Assays (Applied Biosystems, Rotkreuz, Switzerland). Allele-specific polymerase chain reaction (PCR) was performed on a TaqMan thermal cycler (ABI

PRISM® 7900HT system; Life Technologies, Zug, Switzerland). The reaction volume contained 20 ng genomic DNA, 4 µl TaqMan Universal Master Mix (Applied Biosystems, Rotkreuz, Switzerland), 4 µl 20X SNP Genotyping Assay Mix, and 1.6 µl distilled H_2O. Annealing temperature was set to 60°C. After running the PCR, an end‑point fluorescence measurement with the SDS 2.2 software package (Applied Biosystems, Rotkreuz, Switzerland) was obtained, to examine the samples and discriminate between the specific alleles. All genetic analyses were replicated at least once for independent confirmation of the results.

HPLC assessment of salivary caffeine

After thawing, saliva was extracted from the Salivette® according to the manufacturer's instructions (centrifugation for 2 minutes at 1,000g). Salivary caffeine and paraxanthine (caffeine's major metabolite) concentrations were quantified by high performance liquid chromatography (HPLC) coupled to a UV detector, essentially as described by Fuhr and Rost (1994) with minor modifications as follows. The HPLC system consisted of a separations module equipped with a temperature-controlled autosampler (Alliance e2695 XC Separations Module, Waters, Dättwil, Switzerland) and a photodiode array UV detector (2998 PDA Detector, Waters). To summarize, a 225µl aliquot of saliva was prepared by addition of 75µl of trichloroacetic acid 20% containing the internal standard (100mg/l hydroxyethyltheophylline). After vortex mixing and centrifugation (2000g for 10 minutes at +4°C), 20µl of the supernatant were injected onto a Nucleosil 100 C18 reverse phase column (column dimensions 125 x 4mm; 5µm particle size; Macherey-Nagel, Oensingen, Switzerland) and eluted using a 4mmol/l acetic buffer (pH 4.0) containing 1% of acetonitrile, 1% of methanol, and 1.6% of tetrahydrofurane (v/v). The initial flow was increased from 0.8ml/min to 1.0ml/min within 2 minutes, and then kept stable at 1.0ml/min for 17 min, before initial conditions were restored after 2 additional minutes. The samples were usually analyzed in sets of twenty-five, with a calibration row before each set of participants' samples, and a blank sample every ten unknown. Calibration

was based on peak area ratios of paraxanthine and caffeine, respectively, over internal standard for ultraviolet absorption at 273 nm and data point weighting by the inverse of concentrations. The lower limit of quantification (LLOQ) was 0.077 µg/ml for caffeine and 0.024ug/ml for paraxanthine. Precision and accuracy were not more than 14% and 10%, respectively, for the entire concentration range (0.077 – 15.462 µg/ml for caffeine and 0.024 – 12.624 µg/ml for paraxanthine).

Results below the quantifiable limit (BQL) for both caffeine and paraxanthine were assumed to reflect zero caffeine (0 µg/ml). Results that were BQL for caffeine, but quantifiable for paraxanthine, were assumed to reflect half the LLOQ for caffeine (0.077/2 = 0.039 µg/ml).

Legends to Supplementary Figures

Figure S1: Comparison of HPLC-determined salivary concentrations of caffeine and survey estimates of habitual caffeine intake. Pearson's product-moment correlation: $r = 0.317$. Significance (2-tailed): $p < 0.001$. N=326.

Figure S2: Habitual caffeine intake of type 2 diabetes and non-type 2 diabetes groups by dietary category. Boxplots represent self-reported caffeine intake split into separate dietary categories (box: 25^{th} percentile, median and 75^{th} percentile; whiskers: 10^{th} to 90^{th} percentiles; dots: individual data points outside of the whisker range). The estimates of caffeine consumption were based on the caffeine content reported by manufacturers of Swiss products or the website 'Caffeine Informer.' (See Supplementary Table 1). Statistics compared type 2 diabetes (n=134) and Non-type 2 diabetes (n=230) groups. If normally distributed data was available, independent samples t-test was used; if data was not normally distributed, Mann-Whitney U test was used. [**p < 0.001; *p = 0.001. Coffee: independent samples t-test on square-root data (2-tailed). Soft drinks: Mann-Whitney U test on raw data (exact; 2-tailed). Chocolate: independent samples t-test on Log10 data (2-tailed)].

Figure S1

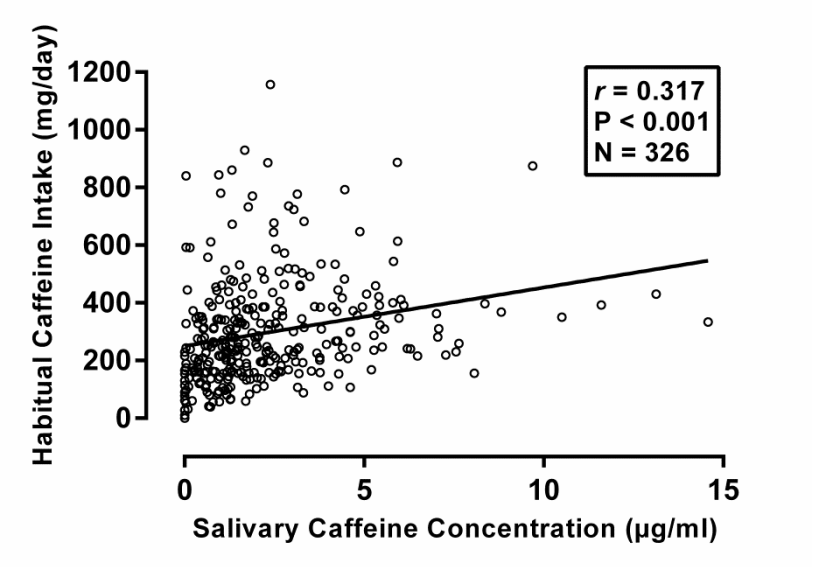
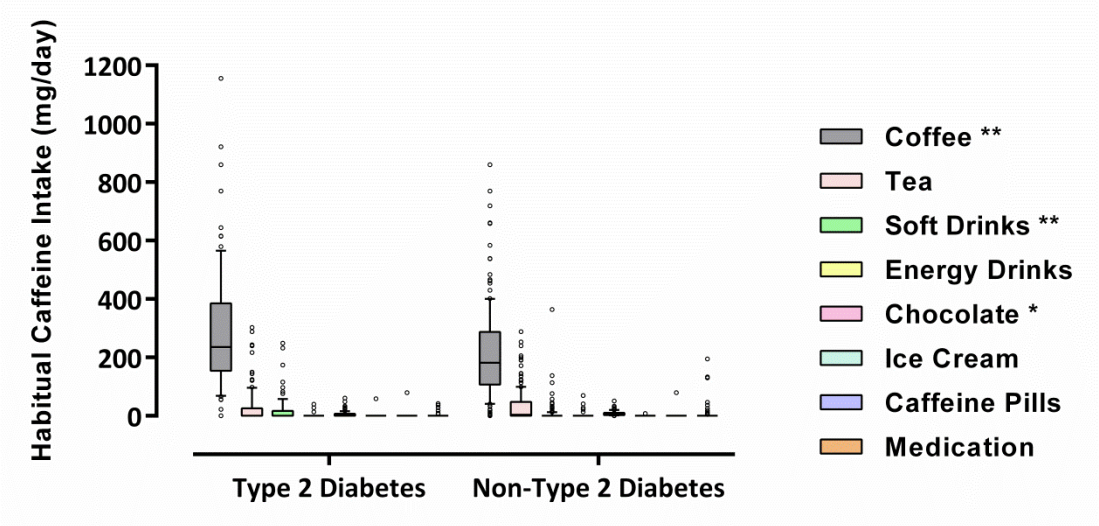

Habitual Caffeine Intake (mg/day)
1200
1000
800
600
400
200
0
r = 0.317
P < 0.001
N = 326
0
5
10
15
Salivary Caffeine Concentration (µg/ml)

Figure S2


Habitual Caffeine Intake (mg/day)
1200
1000
800
600
400
200
0
Type 2 Diabetes
Non-Type 2 Diabetes
Coffee **
Tea
Soft Drinks **
Energy Drinks
Chocolate *
Ice Cream
Caffeine Pills
Medication