Aqueous extracts of Turkish and instant coffees increased glucose level in bovine skeletal muscle tissue in vitro

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This study reported the investigation of possible effects of aqueous coffee extracts on skeletal muscle glucose metabolism. Bovine skeletal muscle homogenate was used in the assays. After adding glucose solution, homogenate was incubated with the extracts and then glucose concentrations were measured at intervals of 2 h. Coffee extracts caused significant increases in skeletal muscle glucose concentrations after 2 h of incubation. The increase was higher in the instant coffee group relative to Turkish coffee group. In the instant coffee group, glucose concentrations were still high at the 4th h, but in the Turkish coffee group was significant decreases at the 4th h. These results show that both Turkish coffee and instant coffee stimulate glycogen breakdown in bovine skeletal muscle tissue, thereby increasing glucose levels in the muscle tissue during the 2 h period. Glucose level in muscle tissue decreased with time possibly because of the fact that tissue glycogen was depleted.

INTRODUCTION

Coffee is a major source of caffeine and chlorogenic acid (Clifford, 2000), and it contains substantial amounts of magnesium and other micronutrients. Several studies have shown that caffeine acutely decreases sensitivity to insulin (Keijzers et al., 2002). The phenol chlorogenic acid in coffee reduces glucose absorption and oxidative stress in vitro (Clifford, 2000) and inhibits hydrolysis of glucose-6-phosphate, which could reduce glucose output in the liver (Arion et al., 1997). Additionally, magnesium intake could improve insulin sensitivity and secretion, which has been associated with a lower risk of type 2 diabetes (Salmeron et al., 1997). In a study, it has been reported that increased coffee consumption for 14 days reduces fasting plasma glucose significantly (Naismith et al., 1970). In another study, it has been established that coffee and caffeine intake might significantly reduce the incidence of type 2 diabetes mellitus (Jiang et al., 2013). O'Keefe et al. (2013) argued that from a cardiovascular (CV) standpoint, coffee consumption may reduce the risk of type 2 diabetes mellitus and hypertension (Zaharieva and Riddell, 2013). Helpful effects of caffeine in the regulation of glucose haemostasis are recently reported by several researchers (Zaharieva and Riddell, 2013; Abrahão et al., 2013; Doo et al., 2013).

However, there are diverging results among the studies as to the role of caffeine in the glucose metabolism. Some of the explanations for the different findings could be that the potentially negative effects exerted by coffee consumption could be offset by protective factors. For example, a recent study from the Netherlands reported that coffee consumption was associated with a substantially lower risk of type 2 diabetes (van Dam and Feskens, 2002). Although the study was large with over 300 new cases of diabetes, it is clear that unexpected
findings of this nature need confirmation from other study populations. Rapid responses to the paper reported diverging findings, with no association between coffee and future diabetes in a Finnish population (Reunanen et al., 2003). In another study from Japan, it was reported that coffee intake was associated with a lower rate of prevalent hyperglycaemia in men and women (Isogawa et al., 2003). It seems that the subject needs further studies with regard to metabolic effects of coffee ingredients in the body. Since skeletal muscle tissue has an important role in the body energetics, investigation of possible effects of coffee ingredients on glucose metabolism in skeletal muscle tissue seems noteworthy.

MATERIALS AND METHODS

Coffee extracts obtained from natural coffee granules (Turkish coffee) and from an instant coffee were prepared by soaking them (10%, w/v) in destilled water and were kept for 24 h at room temperature under constant rotation. After the debris was removed, the extracts were centrifuged at 10,000 rpm for 20 min and the upper hydrophilic clear part was taken and used as the extract in the assays. Bovine skeletal muscle tissue from a male calf was obtained after the animals were sacrificed at slaughterhouse. Tissue samples were kept on ice bath during transportation to the laboratory, which took approximately 1 h. The samples were immediately prepared for the study in the laboratory. All the experiments were performed at 4°C. After Homogenization in physiological saline solution (5%, w/v), skeletal muscle tissue homogenate was centrifuged at 5000 rpm for 10 min to obtain supernatant fractions. Protein concentration of supernatant fraction was 5.6 mg/ml. Assays were performed in this fraction (Durak et al., 2007).

Glucose solution (100 g/100 ml water) was added to the supernatant (Final glucose concentration was 17.2±1.9 mg/dl) and the mixture incubated with changing concentrations (5, 10 and 15 %, w/v) of Turkish coffee and instant coffee extracts for 2 and 4 h. Glucose concentrations were measured at the beginning and after 2 and 4 h incubation periods, respectively. A control assay was also performed, in which no extract was used.

Glucose measurement was performed by using classical folin-Wu method (Folin and Wu, 1920). This method is based on reduction power of glucose, which converts Cu$^{2+}$ to Cu$^{1+}$. During this period, color change was followed by a spectrophotometer. Reduction magnitude observed in color is proportional to the amount of glucose, which is calculated by comparing with the absorbance of a standard glucose solution.

Statistical analysis

Statistical evaluation was done with student’s t-test by using Statistical Package for the Social Science (SPSS).

RESULTS

The results obtained are shown in Tables 1 and 2. As seen from the tables, both coffee extracts caused significant increases in glucose concentrations in skeletal muscle after 2 h of incubation. Values in the instant coffee group were highest. In this group, glucose concentrations were found to be still high at the 4th h relative to the beginning level.

In the Turkish coffee group, glucose levels at the 4th h of incubation were lower than those at the starting levels, but decreases in glucose concentrations were lower when compared with that of control experiment. Because of the increases in glucose levels, which were caused by coffee ingredients, glucose utilisation rates were negative in the coffee groups during the first 2 h (0-2 h) period. However, as seen from the Table 2, glucose utilisation rates were positive at the second 2 h (2-4 h) period. In summary, the glucose utilisation rates were lower in the coffee groups during the 0-4 h period.

DISCUSSION

Several large prospective studies have reported an inverse association between coffee consumption and risk of type 2 diabetes mellitus (Thelle, 1983; Urgert and Katan, 1997; El-Khairy et al., 1999; De Bree et al., 2002). A recent systematic review on this topic concluded that this association between coffee intake and risk of type 2 diabetes mellitus is consistent (LeGrady et al., 1987) and relatively strong, followed a dose-response relation. Furthermore, it has been supposed that it is independent of potentially confounding dietary and lifestyle factors. Purported mechanisms include effects on insulin sensitivity and/or insulin secretion from a variety of minerals, antioxidants, and phytochemical compounds found in coffee (Tverdal et al., 1990; Klag et al., 1994).

However, several researchers argue that although the inverse association between coffee consumption and risk of diabetes has been reported many times, it is still unclear whether the caffeine itself may increase or decrease the risk of type 2 diabetes mellitus (Shino et al., 2010).

Our results show that skeletal muscle glucose metabolism is significantly effected by coffee ingredients. It is possible that caffeine in coffee stimulates glycogen usage, thereby increasing glucose level in the skeletal muscle tissue at the beginning of the 2nd h. Then glucose concentrations began to reduce as glycogen stock depleted; and glucose was used in glycolytic pathway. Aconsideration of the results obtained revealed that the effect of instant coffee on glycogen breakdown is more
drastic than that of Turkish coffee because skeletal muscle glucose levels at the 2nd and 4th h were higher in instant coffee study than those of Turkish coffee. The values of instant coffee group were quite higher at the 4th h as well, relative to Turkish coffee study. It seems that the ingredients of both coffees increased glucose levels in skeletal muscle tissue, thereby causing calculations of false negative glucose utilisation rates. In fact, glucose utilisation rates are not possibly diminished, but increases in glucose concentrations during the experimental period (caused by caffeine-induced glycogen breakdown) led to false reductions in glucose utilisation rates.

Several mechanisms were supposed for the antidiabetic potency of coffee components. In a study, it has been supposed that coffee intake may be associated with reduced risk of type 2 diabetes mellitus because of minerals, phytochemicals and antioxidants in coffee, but the role of caffeine is unclear (Mark et al., 2006). In fact, several plausible mechanisms for a beneficial effect of coffee on glucose metabolism may exist. Coffee has been shown to be a major contributor to the total *in vitro* antioxidant capacity of the diet (Svilaas et al., 2004; Pulido et al., 2003), which may be relevant as oxidative stress can contribute to the development of type 2 diabetes (Ceriello and Motz, 2004). Coffee is the major source of the phenol chlorogenic acid. Intake of chlorogenic acid has been shown to reduce glucose concentrations in rats (Andrade-Cetto and Wiedenfeld, 2001; Rodriguez de Sotillo and Hadley, 2002) and intake of quinides, degradation products of chlorogenic acids, increased insulin sensitivity in rats (Shearer et al., 2003). Chlorogenic acid contributes to the antioxidant effects of coffee (Clifford, 2000) and may reduce hepatic glucose output through inhibition of glucose-6-phosphatase (Arion et al., 1997). It may also improve tissue mineral distribution through its action as a metal chelator (Rodriguez de Sotillo and Hadley, 2002). In addition, chlorogenic acid acts as a competitive inhibitor of glucose absorption in the intestine (Clifford, 2000). Indeed, decaffeinated coffee seemed to delay intestinal absorption of glucose and increased glucagon-like peptide-1 concentrations in an intervention study in humans (Johnston et al., 2003). Glucagon-like peptide-1 is well known for its beneficial effects on glucose-induced insulin secretion and insulin action (Drucker, 1998). This effect may explain the observation that higher coffee

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### Table 1. Values of glucose concentrations (mg/dl) in bovine skeletal muscle tissue homogenates at the beginning, 2 and 4 h later with and without coffee extracts.

<table>
<thead>
<tr>
<th>Homogenates</th>
<th>0 h</th>
<th>2nd h</th>
<th>4th h</th>
</tr>
</thead>
<tbody>
<tr>
<td>H+saline</td>
<td>61.6±7.2</td>
<td>55.3±6.8</td>
<td>44.5±4.5</td>
</tr>
<tr>
<td>H+Turkish coffee extract-1</td>
<td>70.1±8.2</td>
<td>83.8±9.2*</td>
<td>59.9±7.1*</td>
</tr>
<tr>
<td>H+Turkish coffee extract-2</td>
<td>59.9±7.3</td>
<td>68.4±8.0</td>
<td>58.1±6.5</td>
</tr>
<tr>
<td>H+Turkish coffee extract-3</td>
<td>44.9±5.6*</td>
<td>50.2±6.4</td>
<td>44.5±5.4</td>
</tr>
<tr>
<td>H+Instant coffee extract-1</td>
<td>196.1±23.2*</td>
<td>220.6±25.6*</td>
<td>210.3±23.1*</td>
</tr>
<tr>
<td>H+Instant coffee extract-2</td>
<td>150.4±16.5*</td>
<td>169.3±19.0*</td>
<td>148.8±16.8*</td>
</tr>
<tr>
<td>H+Instant coffee extract-3</td>
<td>79.5±7.5</td>
<td>88.9±11.3</td>
<td>72.4±8.8</td>
</tr>
</tbody>
</table>

Values are given in Mean±SD. H, bovine muscle homogenate; extract-1, 25% (w/v) in final volume; extract-2, 15% (w/v) in final volume; extract-3, 5% (w/v) in final volume. *, P<0.05 is significant statistically (wilcoxon non-parametric test).

### Table 2. Rate of glucose utilisation in bovine skeletal muscle tissue with and without tea extracts (n=5).

<table>
<thead>
<tr>
<th>Homogenates</th>
<th>0-2 h</th>
<th>2-4 h</th>
<th>0-4 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>H+saline</td>
<td>31.5±3.2</td>
<td>54±6.1</td>
<td>42.8±5.3</td>
</tr>
<tr>
<td>H+Turkish coffee extract-1</td>
<td>-68.5±7.8*</td>
<td>119.5±13.2*</td>
<td>25.5±3.3*</td>
</tr>
<tr>
<td>H+Turkish coffee extract-2</td>
<td>-42.5±5.4*</td>
<td>51.5±6.4</td>
<td>4.5±5.6*</td>
</tr>
<tr>
<td>H+Turkish coffee extract-3</td>
<td>-26.5±3.1*</td>
<td>28.5±3.0*</td>
<td>1±0.2*</td>
</tr>
<tr>
<td>H+Instant coffee extract-1</td>
<td>-122.5±13.3*</td>
<td>51.5±6.6</td>
<td>-35.5±4.5*</td>
</tr>
<tr>
<td>H+Instant coffee extract-2</td>
<td>-94.5±10.2*</td>
<td>102.5±12.0*</td>
<td>4±0.8*</td>
</tr>
<tr>
<td>H+Instant coffee extract-3</td>
<td>-47±6.0*</td>
<td>82.5±9.8*</td>
<td>17.6±1.5*</td>
</tr>
</tbody>
</table>

Values are given in mean±SD. H, bovine muscle homogenate; extract-1, 25% (w/v) in final volume; extract-2, 15% (w/v) in final volume; extract-3, 5% (w/v) in final volume. *, P<0.05 is significant statistically (wilcoxon non-parametric test).
consumption was associated with lower postload, rather than fasting, glucose concentrations (van Dam et al., 2004; Soriguer et al., 2004; Greer et al., 2001; Yoshioka et al., 2003). In a study, it was reported that, of the two α isoforms of 5’AMP-activated protein kinase (AMPK), AMPKα1 is predominantly activated by caffeine via an energy-independent mechanism and that the activation of AMPKα1 increases glucose transport and ACC phosphorylation in skeletal muscle (Egawa et al., 2011). In another study, Matsuda et al. (2011) demonstrated that coffee and caffeine exerted an ameliorative effect on high-fat-diet-induced impaired glucose tolerance by improving insulin sensitivity and they concluded that this effect might be attributable in part to the reduction of inflammatory adipokine expression. However, Hätönen et al. (2012) argued that coffee does not modify postprandial glycaemic and insulinaemic responses induced by carbohydrates (Hätönen et al., 2012).

In deed, caffeine ingestion can acutely reduce glucose storage (Greer et al., 2001), but beneficial effects of caffeine on lipid oxidation and uncoupling protein-3 expression have also been suggested (Yoshioka et al., 2003). In a study, decaffeinated coffee consumption was inversely associated with risk of type 2 diabetes. In addition, in a Japanese study, the inverse association with hyperglycemia was found to be stronger for coffee than for caffeine (Isogawa et al., 2003). These observations suggest that coffee components other than caffeine may have beneficial effects on risk of type 2 diabetes. Coffee also contains substantial amounts of magnesium, which has been linked to better insulin sensitivity and insulin secretion (de Valk, 1999). However, adjustment for magnesium intake does not explain the association between coffee consumption and risk of type 2 diabetes (van Dam et al., 2004; Salazar-Martinez et al., 2004).

It was concluded from the results of the present study that hydrophilic coffee ingredients (phenolic components like caffeine) cause increases in glucose levels at the beginning in the skeletal muscle tissue through stimulation of glycogen breakdown, which was more marked in the instant coffee. Glucose level in muscle tissue decreased with time possibly because of two reasons: Firstly, consumption of glycogen deposit and secondly, use of glucose in glycolytic pathway.

REFERENCES


