Bezafibrate Mildly Stimulates Ketogenesis and Fatty Acid Metabolism in Hypertriglycerideremic Subjects

Jennifer Tremblay-Mercier, Daniel Tessier, Mélanie Plourde,1 Mélanie Fortier, Dominique Lorrain, and Stephen C. Cunnane

Research Center on Aging, Health and Social Services Center, University Institute of Geriatrics of Sherbrooke, Sherbrooke, Quebec, Canada (J.T.-M., D.T., M.P., M.F., D.L., S.C.C.); and Departments of Medicine (D.T., S.C.C.), Psychology (D.L.), and Physiology and Biophysics (S.C.C.), Université de Sherbrooke, Sherbrooke, Quebec, Canada

Received February 3, 2010; accepted April 16, 2010

ABSTRACT

Our objective was to determine whether bezafibrate, a hypotriglycerideremic drug and peroxisome proliferator-activated receptor (PPAR)-α agonist, is ketogenic and increases fatty acid oxidation in humans. We measured fatty acid metabolism and ketone levels in 13 mildly hypertriglyceridemic adults (67 ± 11 years old) during 2 metabolic study days lasting 6 h, 1 day before and 1 day after bezafibrate (400 mg of bezafibrate per day for 12 weeks). β-Hydroxybutyrate, triglycerides, free fatty acids, fatty acid profiles, insulin, and glucose were measured in plasma, and fatty acid β-oxidation was measured in breath after an oral 50-mg dose of the fatty acid tracer [U-13C]linoleic acid. As expected, 12 weeks on bezafibrate decreased plasma triglycerides by 35%. Bezafibrate tended to raise postprandial β-hydroxybutyrate, an effect that was significant after normalization to the fasting baseline values (p = 0.03). β-Oxidation of [U-13C]linoleic acid increased by 30% (p = 0.03) after treatment. On the metabolic study day after bezafibrate treatment, postprandial insulin decreased by 26% (p = 0.01), and glucose concentrations were lower 2 to 5 h postprandially. Thus, in hypertriglyceridemic individuals, bezafibrate is mildly ketogenic and significantly changes fatty acid metabolism, effects that may be linked to PPARα stimulation and to moderately improved glucose metabolism.

As the population ages, the prevalence of cognitive impairment in the elderly, particularly Alzheimer’s disease (AD), is increasing markedly. Glucose is the brain’s main energy substrate, providing energy to make ATP to maintain ion gradients, synaptic transmission, protein synthesis (for review, see Hoyer, 1996), and fatty acid turnover in membranes phospholipids (Rapoport et al., 2001). A decline in cerebral glucose metabolism is widely reported in AD (Foster et al., 1984; Blennow et al., 2006; Mosconi et al., 2007). This deterioration in brain fuel supply is progressive, correlates broadly with dementia severity, and may appear during normal aging at which time there can be an approximately 6% reduction in glucose uptake per decade (Petit-Taboué et al., 1998). Reduced brain glucose metabolism can arise before the clinical symptoms of AD (Reiman et al., 2004) and may contribute to neurodegenerative processes leading to AD (Liu 2004, 2008). As such, an alternative brain fuel to glucose may be therapeutically useful in AD.

Physiologically, ketone bodies (or ketones; β-hydroxybutyrate (β-OHB), acetoacetate, and acetone) are the main alternative energy substrate to glucose for brain metabolism and are especially important during periods of glucose deprivation. Ketones can supply up to two thirds of the energy requirements of the brain during starvation (Owen et al., 1967). Milder increases in ketone synthesis have been proposed as a possible therapeutic approach to correct or bypass brain

ABBREVIATIONS: AD, Alzheimer’s disease; β-OHB, β-hydroxybutyrate; MCT, medium-chain triglyceride(s); PPAR, peroxisome proliferator-activated receptor; TG, triglyceride(s); AST, aspartate aminotransferase; ALT, alanine aminotransferase; HOMA-IR, homeostasis model assessment-insulin resistance; HDL, high-density lipoprotein; LDL, low-density lipoprotein; FFA, free fatty acid(s); AUC, area under the curve.
glucose hypometabolism associated with AD (Reger et al., 2004; Henderson, 2008). Ketones also have neuroprotective properties in cellular and animal models of neurodegenerative disorders, including AD (Henderson, 2008). Ketones are generated by hepatic fatty acid β-oxidation when plasma glucose and insulin are low (Fukao et al., 2004). The liver cannot use ketones for its own energy requirements, so they diffuse into the blood and reach extrahepatic tissues such as the brain that, unlike other organs, cannot use fatty acids when glucose is limited.

With regular meals, ketogenesis is minimal but it can be stimulated by fasting, administration of a very high-fat ketogenic diet, or ingestion of medium-chain triglycerides (MCT). Ingestion of a drink containing MCT raises plasma β-OHB and is associated with better performance on cognitive tests in AD (Reger et al., 2004; Henderson et al., 2009) and in experimental hypoglycemia in type 1 diabetics (Page et al., 2009). One possible method to induce mild ketonemia without markedly changing the habitual diet is to target the peroxisome proliferator-activated receptor (PPAR)-α (Cullingford, 2004), which is a central nuclear receptor in lipid homeostasis (Isseman and Green, 1990) and is expressed in hepatocytes and astrocytes (Edmond et al., 1987). PPARα stimulation up-regulates genes implicated in lipid transport, lipolysis, fatty acid oxidation, ketone synthesis, and ketone transport (Cullingford et al., 2002; Chapman, 2003).

Drugs of the fibrate class are selective synthetic ligands and agonists of PPARs. Bezafibrate (C_{19}H_{29}CINO_4) is a second-generation fibrate and is mostly used clinically to treat hypertriglyceridemia. Clofibrate, the first fibrate, stimulates ketogenesis in perfused rat liver and increases expression of hydroxymethylglutaryl CoA synthase, a key enzyme in ketogenesis (Mannaerts et al., 1978; Yamamoto et al., 1996; Cullingford et al., 2002, 2004), but nothing is known about whether bezafibrate may also be ketogenic in humans. In the present study, our objective was therefore to determine whether bezafibrate could safely raise fasting, postprandial plasma ketones, or both in humans. We hypothesized that due to an increase in lipolysis, triglyceride (TG) clearance, and fatty acid β-oxidation, bezafibrate would enhance the capacity of the liver to produce ketones. An oral dose of uniformly carbon-13-labeled linoleic acid ([U-13C]18:2n-6) was used to measure fatty acid oxidation before and after bezafibrate.

Materials and Methods

Participant Selection and Characteristics. Participants were recruited based on the presence of mildly elevated fasting plasma TG at the prescreening visit; TG ≥1.5 mM permitted the clinical use of bezafibrate as a hypotriglyceridemic prescription. For inclusion in the study, participants needed to be 50 years old or older, nonsmokers, with no dementia (Mini Mental State Examination, ≥26/30), and in relatively good health. Potential subjects were considered for inclusion if, at the prescreening visit, they had values in the normal range for glucose, hemoglobin A_1C, hepatic enzymes [aspartate aminotransferase (AST), alanine aminotransferase (ALT)], serum creatinine, creatinine clearance, thyroid-stimulating hormone, C-reactive protein, blood cell count, and electrolyte profile. A short questionnaire was used to confirm the absence of alcohol or drug abuse; no use of monoamide oxidase inhibitor-based antidepressants, anticoagulants, or other lipid-lowering therapy; no change in diabetic medication in the previous 12 weeks; and no known liver disease.

Approval for the study was obtained from the Research Ethics Committee of the Health and Social Services Center, University Institute of Geriatrics of Sherbrooke (Sherbrooke, QC, Canada), which oversees all human research done at the Research Center on Aging. All participants gave informed, written consent before enrolment. Thirty-three participants completed the prescreening visit, of which 15 met the inclusion criteria. Complete results were obtained for 13 participants. They were an average of 67 ± 11 years old and their body mass index was 29 ± 5. All patients were cognitively normal (Mini Mental State Examination, 29 ± 1) and had mild hypertriglyceridemia (fasting plasma TG, 1.54–2.77 mM). Nine of the participants had plasma total cholesterol above the normal range. Four participants were not taking any medication, four were taking an antihypertensive (one on Avapro, two on Norvasc and Apo-Hydro, and one on Verapamil), three were taking antidepressants (two on Paxil and one on Ativan), one had been on thyroid hormone (Synthroid), one used a bone resorption inhibitor (Actonel), and two were taking a nonsteroidal anti-inflammatory drug (Celecoxib). These medications were accepted because they are thought not to interact with fibrate therapy and because subjects on those medications had normal blood chemistry profiles at the prescreening visit. None of the participants had been on fibrate therapy before this study.

Metabolic Study Days, Sample Collection, and Bezafibrate Treatment. After prescreening, eligible participants came to the Research Center for three visits: 1) a baseline metabolic study day (before bezafibrate), 2) a blood sample at 6 weeks to verify the absence of side effects of bezafibrate, and 3) a second metabolic study day at 12 weeks (end of bezafibrate). For each visit, they fasted overnight for 12 h. On the two metabolic study days, a forearm venous catheter was installed to draw blood. Before providing the tracer, a breath sample was acquired using a breath collection device (EasySampler; QuinTron Instrument Company, Milwaukee, WI). A breakfast consisting of one to two eggs, three slices of bacon, one slice of toast, and one tomato slice was served to each participant. Fifty milligrams of [U-13C]18:2n-6 (chemical purity, 95%; isotopic purity, 98%; Cambridge Isotope Laboratories, Inc., Andover, MA) was added onto the toast. After breakfast, breath samples were taken every half-hour and blood samples every hour, for 6 h. Only water was permitted during the study day. Blood samples were transferred immediately to a 5-mL tube coated with K$_3$-EDTA (BD Biosciences, Franklin Lakes, NJ) and kept on ice until the end of the study day when they were centrifuged at 2300g for 18 min at 4°C. Plasma was stored at −20°C until further analysis.

Participants took bezafibrate (Bezalip SR; Roche, Auckland, New Zealand) as a single 400-mg tablet once daily for 12 weeks, beginning the day after the baseline metabolic study day. Participants received sufficient bezafibrate for the first 6 weeks; the second batch for the remaining 6 weeks was given after verifying that there were no side effects, as evaluated by blood AST and ALT, which were our measures of liver function.

Plasma Analyses. Commercially available reagent kits were used for the analysis of plasma TG and glucose (Siemens Medical Solutions USA, Inc., Deerfield, IL), free fatty acids (Wako Diagnostics, Richmond, VA), and β-OHB (RX Daytona; Randox Laboratories Ltd., Antrim, UK) using an automated clinical chemistry analyzer (Dimension Xpand Plus; Medical Solutions USA, Inc.). Plasma insulin was analyzed by enzyme-linked immunosorbent assay (Alpco Diagnostics Ltd., Salem, NH) with a microplate reader (model 3550; Bio-Rad Laboratories, Hercules, CA). According to homeostasis model assessment (HOMA), insulin resistance (IR) was calculated as fasting plasma glucose (millimoles per liter) × fasting plasma insulin (microunits per milliliter)/22.5. Fatty acid profiles of plasma total lipids were analyzed by capillary gas chromatography, as described previously (Plourde et al., 2009). In brief, plasma lipids were extracted into chloroform/methanol (2:1) (Folch et al., 1957). Total lipids were then saponified with 1 M KOH-methanol, and free fatty acids were methylated with 14% boron trifluoride-methanol (Sigma
TABLE 1

<table>
<thead>
<tr>
<th>Plasma Lipids</th>
<th>Before</th>
<th>After</th>
<th>%Δ*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglycerides (mM)</td>
<td>2.0 ± 0.9</td>
<td>1.3 ± 0.5**</td>
<td>−35</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>6.0 ± 0.8</td>
<td>5.0 ± 0.5*</td>
<td>−17</td>
</tr>
<tr>
<td>Cholesterol-HDL (mM)</td>
<td>1.4 ± 0.4</td>
<td>1.7 ± 0.7*</td>
<td>+21</td>
</tr>
<tr>
<td>Cholesterol-LDL (mM)</td>
<td>3.7 ± 0.6</td>
<td>2.9 ± 0.5*</td>
<td>−22</td>
</tr>
</tbody>
</table>

* p < 0.05; ** p < 0.01.

Percentage of change shown when differences were statistically significant.

---

**Results**

Eight women and five men completed the study. No side effects were reported by the subjects or detected during the blood analyses of AST or ALT (data not shown). Bezafibrate significantly decreased FFA, total cholesterol, and LDL cholesterol by 35, 17, and 22%, respectively, and increased HDL cholesterol by 21% (Table 1). The TG-lowering effect persisted throughout the study (Fig. 1), but postprandial TG response day as measured by the AUC of the normalized TG data did not change after bezafibrate (data not shown).

Bezafibrate did not change plasma ß-OHB concentration significantly at any time during the final metabolic study day, although there was a trend toward an increase at 6 h (p = 0.075; Fig. 2A). However, bezafibrate did significantly increase the AUC of the postprandial ß-OHB response by 58%, and after normalization to baseline, it also increased the ß-OHB response at 4 and 5 h (Fig. 2B). The AUC of the ß-OHB response tended to be inversely related to the decrease in plasma TG (r = −0.545; p = 0.067; data not shown).

After bezafibrate, the plasma FFA response was lower at 2 and 6 h during the study day (−14 and −11%, respectively), and the AUC of the postprandial response tended to decrease (p = 0.055; Fig. 3A). [U-13C]Linoleate ß-oxidation peaked at approximately 5 h (Fig. 3B) and returned to baseline 24 h after ingestion both before and after bezafibrate (data not shown). After treatment, the rate of linoleate ß-oxidation (percentage of dose per hour recovered in exhaled CO2) was significantly higher from 3.5 to 4.5 h and at 5.5 h after ingestion, and the AUC was 30% higher (Fig. 3B).

Plasma insulin was lower at 2, 4, 5, and 6 h, and the AUC of the postprandial insulin response was 26% lower after bezafibrate (p = 0.01; Fig. 4A). Plasma glucose was lower 2 to 5 h after bezafibrate (Fig. 4B), but the AUC of the postprandial response was not significantly different (data not shown). The index of insulin resistance calculated by the HOMA-IR revealed a tendency for these values to decrease after treatment; however, statistical significance was not reached (1.81 ± 0.79 versus 1.36 ± 0.49; p = 0.087). The percentage of change in postprandial glucose and ß-OHB responses during the metabolic study day were significantly inversely correlated; thus, as postprandial glucose AUC response decreased after bezafibrate, postprandial ß-OHB AUC response increased (r = −0.72; p = 0.008; Fig. 4C).

All saturated fatty acids, linoleic acid and most ω3 polyunsaturated fatty acids in fasting plasma decreased after beza-
fibrate (Table 2). Total fatty acid concentration (milligrams per liter) decreased by 14% after bezafibrate treatment.

Discussion

Our results showed that bezafibrate increases fatty acid β-oxidation and postprandial plasma ketone β-OHB response in humans. This effect on β-OHB was mild and only seen after normalization to fasting values, in part, because of large variation of β-OHB among our participants. The mild ketogenic effect may be due to reduced plasma glucose and insulin and increased fatty acid β-oxidation. Whether the mild ketogenic effect of bezafibrate is due directly to its hypotriglyceridemic effect remains to be determined; such a possibility seems likely, but the inverse relationship between the level of postprandial ketonemia and the decrease in plasma TG we observed did not quite reach statistical significance.

The metabolic study day before bezafibrate permitted us to examine the relation among insulin, fatty acid oxidation, and ketogenesis. Plasma β-OHB began to increase approximately 3 h after the meal (Fig. 2A), an effect that may be explained in part by decreasing insulin starting approximately 2 h postprandially (Fig. 4A), which coincided with rising plasma FFA (Fig. 3A) and enhanced fatty acid β-oxidation (Fig. 4B). Therefore, we hypothesize that this fall in plasma insulin allowed the release and β-oxidation of FFA and subsequent ketone body production, as suggested previously (McGarry and Foster, 1977; Fukao et al., 2004; Soeters et al., 2009).

Bezafibrate for 12 weeks had significant effects on several outcomes that were followed during the two metabolic study days. First, all participants had lower plasma TG (Fig. 1), consistent with previous studies showing a 30 to 50% decrease in TG while on a fibrate (Chapman, 2003). Because PPARα is a hepatic receptor and we had no access to samples of liver, we do not have a direct measure of PPARα expression or activity. However, our protocol was clearly hypotriglyceridemic, so we speculate that PPARα was activated because this is normally the mechanism whereby bezafibrate reduces plasma TG.

Second, after bezafibrate, insulin response to the breakfast was reduced and glucose decreased most of the time during the metabolic study day, suggesting that PPARα activation may have improved insulin sensitivity. The literature is inconsistent regarding whether fibrates improve glucose homeostasis; some studies showed improved insulin sensitivity (Jonkers et al., 2001; Damci et al., 2003; Kim et al., 2003; Cree et al., 2007; Tenenbaum et al., 2007), but others report that fibrates raise glucose and glycosylated hemoglobin and do not improve insulin sensitivity (Sane et al., 1995; Asplund-Carlson, 1996; Anderlová et al., 2007). Because hyperlipidemia and FFA overload contribute to insulin resistance...
before and after bezafibrate. Numerous studies in a variety of experimental models show that linoleic acid and α-linolenic acid are the most easily β-oxidized of the common dietary fatty acids (for review, see Cunnane et al., 2003), and both these fatty acids decreased after bezafibrate (20 and 24%, respectively; Table 2). De Lorgeril’s team observed the same effect after 12 weeks on fenofibrate (de Lorgeril et al., 2005).

Our study had several limitations. First, because fasting β-OHB was near the lower detection limit and the interindividual variation was considerable (coefficient of variation on fasted plasma of 70%), the sample size was too small to reach statistical power for fasted β-OHB values. Second, participants’ body weights were not systematically measured after bezafibrate treatment; thus, loss or gain in body weight could not be taken in consideration to explain plasma metabolite changes, especially with regard to glucose homeostasis.

Experimental ketonemia of approximately 0.3 to 0.4 mM induced by MCT ingestion has beneficial effects on cognition in AD (Henderson et al., 2009) and in type 1 diabetics during acute hypoglycemia (Page et al., 2009). With bezafibrate, we did not reach this level during fasting, but we did reach 0.3 to 0.4 mM β-OHB at the end of the metabolic day. Perhaps in conjunction with a fructose, joint administration of a low dose of MCT or a relatively easily β-oxidized long-chain fatty acid, i.e., myristic, linoleic, or α-linolenic acids, would make it possible to achieve a more constant plasma β-OHB level of 0.3 to 0.4 mM, while avoiding the common gastrointestinal side effects of MCT alone. Diabetes, insulin resistance, and hyperlipidemia are important risk factors for AD and because bezafibrate has a beneficial impact on insulin sensitivity as well as mild ketogenic potential, fibrates may have potential as pharmacological agents aiming to reduce the risk of AD. Indeed, fructose used tended to be associated with a lower prevalence of dementia (Dufouil et al., 2005) in a large European study based on the Three-City Study; thus, further research into the possible benefits of the mild ketogenic effect of bezafibrate is warranted.

Acknowledgments
We thank the study participants; research nurses Julie Desgagnés and Martine Fisch; and Scott Nugent, Stéphane Protat, and Linda Bergeron.

References
D’Costa MA and Angel A (1975) Inhibition of hormone-stimulated lipolysis by clofi-


Address correspondence to: Jennifer Tremblay-Mercier, Research Center on Aging, 1036 Belvedere St. South, Sherbrooke, QC J1H 4C4, Canada. E-mail: jennifer.tremblay-mercier@usherbrooke.ca