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ARTICLE

An Onion Byproduct Affects Plasma Lipids in Healthy Rats

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Onion may contribute to the health effects associated with high fruit and vegetable consumption. A considerable amount of onion production ends up as waste that might find use in foods. Onion byproduct has not yet been explored for potential health benefits. The aim of this study is to elucidate the safety and potential role of onion byproducts in affecting risk markers of cardiovascular disease (CVD). For that purpose, the effects of an onion byproduct, *Allium cepa* L. *cepa* 'Recas' (OBP), and its two derived fractions, an ethanolic extract (OE) and a residue (OR), on the distribution of plasma lipids and on factors affecting cholesterol metabolism in healthy rats have been investigated. The OBP or its fractions did not significantly reduce cholesterol or down-regulate hepatic 3-hydroxy-3-methylglutaryl-coenzyme A reductase (*Hmgcr*) gene expression. The OR even had the effect of increasing plasma triacylglycerides (TAG) and cholesterol in the very low density lipoprotein (VLDL-C) fraction. Neither total bile acids nor total primary or secondary bile acids were significantly affected by feeding rats the OBP or its fractions. Principal component analysis combining all markers revealed that the controls could be completely separated from OBP, OE, and OR groups in the scores plot and also that OE and OR groups were separated. Plasma lipids and bile acid excretion were the discriminating loading factors for separating OE and OR but also contributed to the separation of onion-fed animals and controls. It was concluded that the onion byproduct did not present significant beneficial effects on individual markers related to plasma lipid transport in this healthy rat model but that onion byproduct contains factors with the ability to modulate plasma lipids and lipoprotein levels.

KEYWORDS: Onion byproduct; CVD risk factors; bile acids; gene expression; plasma lipids; platelet aggregation

INTRODUCTION

The reported health benefits of *Allium* vegetable constituents include cardiovascular effects, improvement of the immune function, lowering of the blood glucose level, radioprotection, protection against microbial infections, and anticancer effects (*1*). Among *Allium* vegetables, most of the studies have focused on garlic (*Allium sativum* L.) and its constituents, particularly organosulfur compounds (OSCs) and their cardioprotective and anticarcinogenic effects (*2*, *3*). Onion (*Allium cepa* L.) is among the most highly consumed vegetables worldwide, and it has been a target of fewer studies despite the fact that this vegetable is one of the food items which have been associated with better survival of heart attacks in observational studies (*4*).

Onion byproduct has been characterized (*5*), and some biological responses of a particular onion byproduct (*Allium cepa* L. *cepa* 'Recas') and two derived onion fractions have been described in our previous investigations (*6*). Moreover, two dietary metabolic onion intake biomarkers have recently been identified (*7*). Taking into account that cardiovascular disease (CVD) is a prevalent disease worldwide, we consider it of importance to study the potential health benefits of an onion byproduct in terms of CVD prevention (*8*).

CVD accounted for about 30% of the 58 million estimated deaths globally from all causes in 2005. Between 2006 and 2015, deaths due to noncommunicable diseases are expected to increase by 17%, of which half will be due to CVD (*9*). The rise in CVD reflects a significant change in dietary habits, physical activity levels, and tobacco consumption worldwide. High blood pressure, high blood cholesterol, overweight, obesity, and type 2 diabetes are among the major biological risk factors. Unhealthy dietary practices may include the high consumption of saturated fats, salt, and refined carbohydrates, as well as low consumption of fruits and vegetables (*10*). Several early risk factors for CVD are known, among them a high plasma level of triacylglycerides (TAG), total cholesterol (TC), cholesterol in the low-density

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lipoprotein fraction (LDL-C) and in the very low density lipoprotein fraction (VLDL-C), and a low level of cholesterol in the high-density lipoprotein fraction (HDL-C). Prevention of CVD by reducing some of the main CVD risk factors represents one of the main targets of preventive nutrition. Despite differences in reverse cholesterol transport between rats and humans, the rat has been useful as a model of cholesterol lowering by dietary fiber due to its sensitivity to bile acid depletion, short-chain fatty acid production, and fructose feeding (*11*, *12*).

Large amounts of byproduct from onion are produced worldwide, but currently these products have limited use. We have previously shown that an onion byproduct could alter the gut environment in the rat and increase the formation of short-chain fatty acids (*6*). To our knowledge no studies have focused on onion byproduct and its potential role as a CVD preventive food ingredient. Therefore, we aimed to elucidate the potential protective role of an onion byproduct in affecting factors related to plasma cholesterol levels in a model study using healthy rats. We also aimed to study which major fiber fractions, that is, soluble or insoluble fiber, of the onion byproduct may be responsible for any effects to shed light on potential bioactive constituents.

MATERIALS AND METHODS

Chemicals. All chemical reagents used were of analytical grade from Fluka (Steinheim, Germany), Merck (Darmstadt, Germany), and Sigma-Aldrich (Brøndby, Denmark). Ethanol (96%) was purchased from De Danske Spritfabrikker, Aalborg, Denmark. Water was Milli-Q (Millipore, Bedford, MA) with >18 Mohm resistivity. The bile acids dehydrocholic acid (DHCA), ^{13}C-glycocholic acid (^{13}C-GCA), ursodeoxycholic acid (UDOCA), chenodeoxycholic acid (CDOCA), and litocholic acid (LCA) were purchased from Sigma-Aldrich Chemicals. The bile acids tauroursodeoxycholic acid (TUDOCA), glycoursodeoxycholic acid (GUDOCA), taurocholic acid (TA), glycocholic acid (GA), taurochenodeoxycholic acid (TCDOCA), cholic acid (CA), taurodeoxycholic acid (TDOCA), glycodeoxycholic acid (GDOCA), and deoxycholic acid (DOCA) were purchased from Merck. The bile acid standards, α-muricholic acid and *β*-muricholic acid, were obtained from Steraloids (Newport, RI).

Onion Byproduct Extraction and Analysis. A freeze-dried onion byproduct powder (OBP) was obtained from an onion byproduct pasteurized paste (*Allium cepa* L. *cepa* 'Recas') at Instituto del Frío-CSIC, Madrid (*5*). Two derived fractions, a 60% ethanolic extract (onion extract, OE) and the resulting dry onion residue (OR), were produced as previously described (*6*) according to a modified Shiomi method as described by Jaime et al. (*13*). About 70% of the OBP could be extracted into the OE fraction, whereas 30% remained afterward as OR. The OE is rich in soluble fiber, whereas the OR is rich in insoluble fiber.

The sample extraction and preparation procedures for each analysis performed are extensively explained in our previous paper (*6*). Briefly, soluble sugars were determined by standard methods (*14*−*16*). Starch was degraded to glucose units as previously described (*17*), and fructans were determined after fructanase treatment according to the protocol of the manufacturer (Megazyme International, Bray, Ireland). All assays were performed in microplates using a Spectra-Max 190 microplate reader (Molecular Devices, Sunnyvale, CA). High-performance anion exchange chromatographic analysis of fructooligosaccharide size distribution was performed as described previously for glucans (*18*). Each onion product was analyzed for quercetin contents. Analysis was performed both with and without preceding hydrolysis of quercetin glucosides (1.2 M HCl at 90 °C for 2 h). Genisten was used as an internal standard. Separation was obtained on a 2.1 mm × 10 cm C_{18} BEH column (1.7 μm particle size) using a UPLC system (Waters, Milford, MA) coupled with a TQD operated in the multiple reaction mode (MRM) for quantitative analysis as described previously (*6*). CV% for all food analyses was better than 5%.

Rat Study Design and Sample Collection. Four groups of healthy male Fisher 344 rats obtained from Charles River (Sulzfeld, Germany) were fed during 4 weeks with either a semisynthetic control diet (group 1, Ctrl), a nutritionally balanced control diet containing 10% of OBP (OBP group), a balanced control diet containing 7% of OE (OE group), or a balanced control diet containing 3% OR (OR group). The amounts of OE and OR added to the feeds correspond to their relative contribution to the OBP starting material. All four diets were based on a semisynthetic rodent diet produced at the National Food Institute, Technical University of Denmark, and were nutritionally balanced with respect to monomeric and polymeric carbohydrates, lipids, protein, and micronutrients as detailed previously (*6*). Animal experiments were carried out under the supervision of the Danish National Agency for the Protection of Experimental Animals. All animal study procedures have been approved by the Institutional Committee for Animal Experimentation, and the National Food Institute has been approved for this type of experiment with rodents by the Danish Ministry of Justice. Feces samples were collected during 24 h two days before the termination of the rat study while the rats were housed singularly in metabolic steel cages with a device to separate urine from feces. The feces was weighed and stored frozen at −80 °C until bile acid analysis. After 4 weeks on the experimental diets, the animals were fasted overnight. The next day the rats were anesthetized in CO_2/O_2 and sacrificed by decapitation. Immediately after the decapitation, blood was collected into Vacutainer tubes containing heparin as an anticoagulant. After 10 min of incubation on ice, the samples were centrifuged at 1500*g* for 10 min at 4 °C. Plasma was removed for analyses of enzymes, triacylglycerides (TAG), and lipoproteins. Rat liver was removed and ground in liquid N_2 to a fine powder. Samples of 30 mg of liver were stored at −80 °C for gene expression analysis.

Excretion of Sulfur Compounds. As a marker of rat urinary concentration of metabolites of sulfur-containing compounds from OBP we have previously reported the identification of dimethyl sulfone by NMR analysis (*7*). The signal intensity recorded at 3.16 ppm was recorded for each rat as a marker of the dimethyl sulfone concentration in urine.

Biochemical Analysis. Markers of Hepatic Function, and TAG. Alkaline phosphatase (AP), alanine aminotransferase (ALAT), *γ*-glutamyl transferase (GGT), and TAG concentrations were measured in rat plasma samples using an automated Roche/Hitachi 912 analyzer at 37 °C in accordance with the instructions of the manufacturers (Roche Diagnostic GmbH, Mannheim, Germany).

RNA Isolation and Quantitative Real-Time PCR. Five liver samples from each group were used for measurement of the expression of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (*Hmgcr*). Relative mRNA expression was quantified by real-time PCR on an ABI 7900HT FAST System as described previously (*6*). Control group samples were pooled and used as a calibrant. TaqMan Gene Expression Assays used were the following: Eukaryotic 18S rRNA Endogenous Control (catalog no. 4352930E); rat *Hmgcr* (catalog no. Rn_00695772_g1).

^{1}H NMR Analysis and Chemometric Models for Lipid Quantification. Total cholesterol and cholesterol content in high, low, and very low density lipoproteins (HDL-C, LDL-C, and VLDL-C) were analyzed in rat plasma samples. For ^{1}H NMR analysis, plasma samples were thawed on ice, 100 μL of plasma was transferred to a 5 mm NMR tube, and 450 μL of D_2O was added. NMR spectra were acquired on a Bruker Avance 400 MHz spectrometer (9.4 T) (Bruker Biospin Gmbh, Rheinstetten, Germany) at 311 K, which corresponds to the body temperature of rats. Total cholesterol and cholesterol contents in HDL, LDL, and VLDL lipoproteins were then predicted by previously developed chemometric models based on NMR data and interval partial least-squares models as described previously (*19*).

Bile Acids Analysis by LC-MS/MS. The concentration of bile acids in feces samples was measured by a novel LC-MS/MS method (Jensen et al., unpublished data). Briefly, total feces was weighed and homogenized with 14 volumes (w/v) of water into a slurry. A weighed aliquot (approximately 0.3 g) of this homogenate was added with the internal standard, ^{13}C-GCA, and extracted once with 100% acetonitrile (ACN) and then twice with 50% ACN. The extract was diluted with 0.1% formic acid and concentrated on an Oasis HLB 3 cm^3 column (Waters). The ACN eluate was evaporated to dryness and redissolved in 15% ACN, 30% methanol, and 0.1% formic acid, giving an overall dilution factor of 2.5. Samples and standards were analyzed on an Acquity UPLC with a TQ detector (Waters), operated in MRM mode with a gradient from phase A to B over 5 min. The mobile phases are 30% methanol−0.1% formic acid (mobile phase A) and 100% ACN−0.1% formic acid (mobile phase B) at a total flow rate of 0.9 mL/min. Between-run CV% for the internal standard

Table 1. Glucose, Fructose, Sucrose, and Fructans Contents in the Onion Byproduct (OBP) and Its Two Derived Onion Fractions, Onion Extract (OE) and Onion Residue (OR)[a]

	glucose (mg/g)	fructose (mg/g)	sucrose (mg/g)	fructans (mg/g)
OBP	205.6 ± 4.9	189.4 ± 9.2	96.9 ± 4.4	42.5 ± 4.8
OE	215.8 ± 4.8	199.3 ± 5.6	85.7 ± 2.1	71.7 ± 8.8
OR	102.8 ± 8.6	95.5 ± 8.6	53.2 ± 3.9	30.8 ± 2.9

[a] Numbers are means ± SD of three determinations based on wet weight. The humidity in each fraction was 17.3, 18.2, and 7.3%, respectively, for the OBP, OE, and OR.

(n = 7) was 4% for ^{13}C-GCA, and the recovery for internal standard throughout the sample workup procedure and analysis was 65 ± 10% (mean ± standard diviation, n = 50). By applying specific transitions, 15 individual bile acids were quantified using QuanLynx version 4.1 (Waters) based on internal standard and external calibrants. On the basis of the analytical results for the individual primary and secondary bile acids, these were summed for each rat to give total primary and total secondary bile acids, respectively. The intraday and interday variations for the individual bile acids varied from 2 to 10% and from 3 to 12%, respectively, during these analyses.

Statistical Analysis. The data were analyzed for normal distribution using the Shapiro−Wilcks W test and for homogeneity of variance using Levene's test (P > 0.05). Plasma TG and LDL cholesterol, plasma GGT activity, and liver HMGCR expression had to be log transformed to meet these criteria. The normally distributed and variance homogeneous data were analyzed by ANOVA. If significant differences were found between groups, further comparisons were done using least-squares means. We used the SAS statistical package v. 9.1 (SAS Institute, Cary, NC) and consider a P value of < 0.05 to be significant. Unsupervised multivariate analysis (principal component analysis, PCA) was carried out with all of the effect variables reported here as well as those reported in our previous publication on toxicity, gut health, and antioxidant parameters (*6*) using Latentix 1.0 (Latent5 Ltd., Copenhagen, Denmark). Gene expression variables were omitted because they were performed for only five animals per group. Variables were mean centered and autoscaled before analysis. Scores plots and loadings plots were generated automatically by the software.

RESULTS

Composition of Onion Byproduct and Its Fractions. Fructose, glucose, sucrose, and fructan contents in the OBP and its fractions are shown in **Table 1**. A semiquantitative size distribution analysis of the fructans in the OE indicated that > 90% had 10 fructose residues or less and > 60% had 5 residues or less. Very small amounts of longer chain fructans were present. Starch was not found in any of the samples. Total quercetin contents in the OBP, OE, and OR after hydrolysis of glycosides were found to be 3.37 ± 0.52, 3.97 ± 0.01, and 1.22 ± 0.33 mg/g of wet weight, respectively.

Excretion of Sulfur-Containing Metabolites. The relative amounts excreted into urine of the major sulfur-containing metabolite, dimethyl sulfone, detected by NMR analysis of the collected urine sample (*7*) as compared with control rats (mean ± SD; 100 ± 11%) was 272 ± 12% in the OBP group, 202 ± 12% in the OE group, and 136 ± 17% in the OR group.

Markers of Liver Function and Gene Expression. The effects of feeding rats OBP on plasma activities of the hepatic enzymes, alanine aminotransferase (ALAT), alkaline phosphatase (AP), and γ-glutamyl transferase (GGT), are shown in **Table 2**. GGT activity was higher in rats fed the OBP or OE compared to the control group. By contrast, ALAT and ALT activities were lower when rats were fed the OBP or its derived fractions (OE and OR) compared to the control group.

Hepatic expression of the gene encoding the rate-limiting enzyme involved in cholesterol biosynthesis, 3-hydroxyl-3-methylglutaryl-coenzyme A reductase (*Hmgcr*), is also shown in **Table 2**. *Hmgcr* was not significantly altered (P = 0.3) as a consequence of feeding with the OBP or either of its fractions (OE and OR).

Table 2. Effects of Feeding Onion Fractions on Rat Plasma Activities of Hepatic Enzymes and on Hepatic Expression of 3-Hydroxy-3-methylglutaryl-coenzyme A Reductase (*Hmgcr*)[a]

rat group[b]	ALAT (UI/L)	GGT (UI/L)	AP (UI/L)	*Hmgcr*[c]
control	91.14 ± 8.21	2.40 ± 0.59	753.57 ± 71.59	0.94 ± 0.69
OBP	85.0 ± 7.97*	3.40 ± 1.43*	693.87 ± 67.22*	0.53 ± 0.51
OE	76.87 ± 8.76*	3.19 ± 1.90*	673.50 ± 75.33*	0.46 ± 0.30
OR	76.37 ± 6.72*	2.67 ± 0.87	663.87 ± 31.10*	0.54 ± 0.23

[a] Results are expressed as mean ± SD; n = 8. *, P < 0.05 as compared with control. ALAT, alanine aminotransferase; GGT, γ-glutamyl transferase; AP alkaline phosphatise. [b] OBP, onion byproduct fed at 10% in the diet; OE, an ethanolic extract of OBP fed at 7% in the diet; OR, the residue from OBP extraction, fed at 3% in the diet. [c] Gene expression (n = 5) of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (*Hmgcr*) is given relative to the endogenous reference 18S rRNA and a calibrant (RQ).

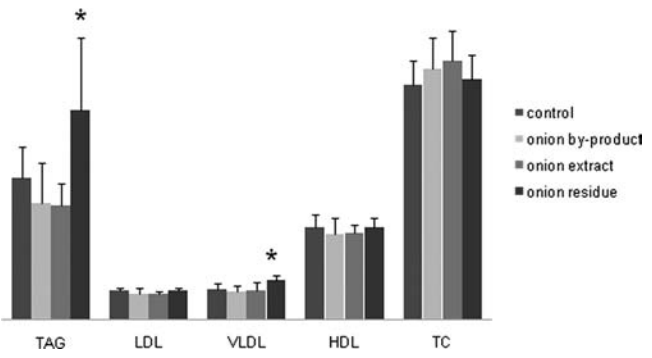

Figure 1. Effects of feeding with onion byproduct (OBP) and its derived fractions, onion extract (OE) and onion residue (OR), on rat plasma triacylglycerides (TAG), total cholesterol (TC), and cholesterol in very low (VLDL-C), low (LDL-C), and high (HDL-C) lipoprotein fractions. Asterisks (∗) on the bars indicate significant difference between the onion groups and the control group at P < 0.05.

Lipids. TAG concentrations, total cholesterol (TC), and cholesterol content in lipoproteins (HDL-C, LDL-C, and VLDL-C) in rat plasma samples are shown in **Figure 1**. The results show that rat plasma TAG and TC concentrations were not significantly altered in healthy, fasted rats fed with the OBP or the OE, although OE had a borderline significant lowering effect on LDL cholesterol (P = 0.06). By contrast, the OR significantly increased TAG (P = 0.001) and VLDL-C (P = 0.016) cholesterol concentration values in the fasting state.

Bile Acids im Feces. The excretion of bile acids in feces of rats fed OBP, OE, or OR is shown in **Table 3**. We determined a total of 15 different bile acids in the collected fecal samples, including the major primary rat bile acids, α- and β-muricholic acid. Neither total bile acids nor total primary or secondary bile acids were significantly affected by feeding the OBP or its fractions (OE and OR), although the excretion of primary bile acids was almost numerically doubled by feeding the OE or the OR, but not by the whole OBP. This apparent increase was caused primarily by doubling the excretion of α- and β-muricholic acids following feeding of the rats with OE or OR. However, the excretion of α-muricholic acid was not significantly different from controls in these groups No other major bile acids nor the ratio between conjugated and unconjugated bile acids was significantly affected by the treatments (data not shown). Fecal output differed considerably between rats from 0.22 to 2.49 g/24 h with no significant difference between the groups (data not shown).

Table 3. Primary and Secondary Bile Acid Excretion in Feces of Rats Fed an Onion Byproduct (OBP) and Its Two Derived Onion Fractions, Onion Extract (OE) and Onion Residue (OR)[a]

rat group	primary bile acids	α-muricholic acid	secondary bile acids
control	0.33 ± 0.24	0.26 ± 0.19	0.15 ± 0.11
OBP	0.32 ± 0.19	0.23 ± 0.18	0.15 ± 0.09
OE	0.64 ± 0.43	0.54 ± 0.36	0.19 ± 0.08
OR	0.77 ± 0.79	0.68 ± 0.65	0.14 ± 0.15

[a] Values are expressed as means ± standard deviations in units of mg/day.

There was no relationship between fecal output and total bile acid excretion.

Multivariate Analysis. PCA of all effect variables in combination leads to a complete separation in the scores plot (**Figure 2A**) of control animals from animals fed any of the onion products using the first two and the fifth principal components (line a in **Figure 2A**). These principal components explain slightly less than half of the total variation in the data. The same three principal components also separate the effects of the OE and OR fractions in a perpendicular direction (line b in **Figure 2A**). Inspection of the loadings plot (**Figure 2B**) shows that three sets of markers explain most of the difference between feeding with any onion products and control. These sets are related either to liver function, to anemia (toxicity markers), or to gut functionality. The latter group of markers is particularly affected by propionate and butyrate production, bile acid excretion, and the glucosidase and glucoronidase enzyme activity of the gut microbiota. The separation of OE and OR in the scores plot is mainly explained by the concentrations of HDL-C, LDL-C, and total cholesterol as seen from the loadings plot.

DISCUSSION

In the current study we report the effects caused by an onion byproduct on total lipids, lipoproteins, bile acids, and some liver function test markers in an animal model using healthy rats. The onion byproduct (OBP) used to feed the rodents in our study offers the technological advantage of having a real possibility for being developed as an antioxidant and antibrowning ingredient in foods (*5*). The safety of adding this onion product is therefore an important aspect of the current study. A fructan and fructo-oligosacharide (FOS) extraction of the OBP was performed with ethanol to yield an OE because one of our aims was to elucidate the role of the soluble and insoluble onion fibers on the selected markers. We previously reported the concentrations of these compounds as well as free sugars, total starch, and quercetin in the OE and ER fractions. It can be calculated that of the total amount of sugars and soluble fibers present in the feed containing OBP, approximately 85−90% is in the feed with onion extract (OE) and the remaining 9−15% is in the feed with onion residue (OR) (**Table 1**). Moreover, analysis of dimethyl sulfone, a sulfur-containing metabolite, in urine from rats fed the OBP and its two fractions (OE and OR) revealed that the starting material for this compound appears to have approximately the same concentration in all three tested products (*7*), but due to differences in the amounts of OBP, OE and OR fed to the rats, the excretion varies accordingly. As detailed here dimethyl sulfone was found in all urine samples, also in controls, and is therefore a metabolite originating from other dietary sources in addition to onion. We hypothesize that its increase originates from onion polysulfide degradation by the gut microflora, but this has to be verified by further research. Asparagus also contains sulfides, and dimethyl sulfone has been reported as a urinary metabolite following asparagus consumption (*20*).

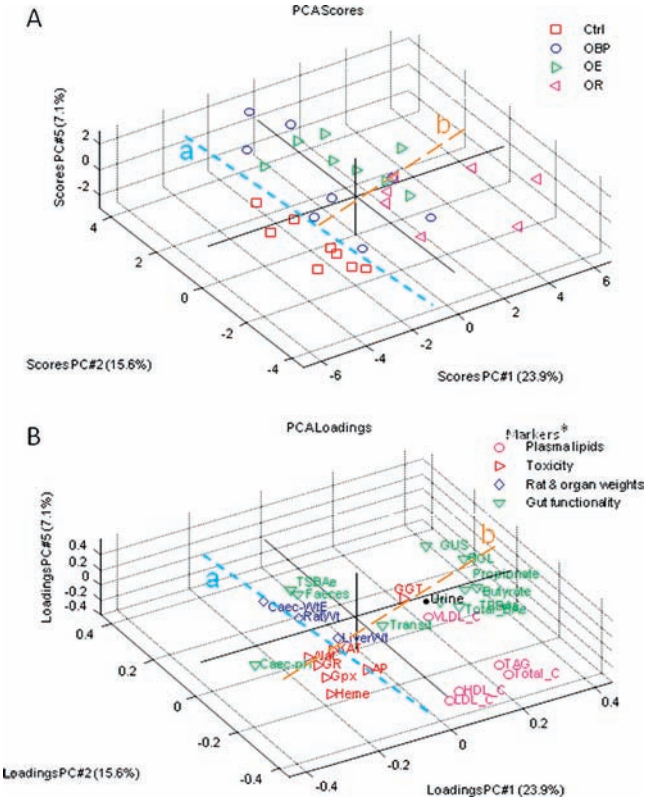

Figure 2. Principal component analysis of effect markers in rats after feeding with onion fractions: scores (**A**) and loadings (**B**) plots from a PCA with markers determined in rats fed an onion byproduct (OBP) or its two fraction, an ethanolic extract (OE) and the residue (OR) after extraction, as compared with control rats (Ctrl). Abbreviations: Alat, plasma alanine aminotransferase; AP, plasma alkaline phosphatise; BGL, cecum β-glucuronidase; Cec-WtE, cecum weight (empty); Faeces, total 24 h fecal excretion; GGT, plasma γ-glutamyl transpeptidase; Gpx, erythrocyte glutathione peroxidase activity; GR, erythrocyte glutathione reductase activity; GUS, cecum β-glucosidase activity; Heme, erythrocyte total hemoglobin; HDL_C, plasma HDL cholesterol; KAT, erythrocyte catalase activity; LDL_C, plasma LDL cholesterol; Liver-Wt, total liver wet weight; Rat-Wt, total rat weight at sacrifice; Total_BAe, total bile acid excretion; TBPAe, total primary bile acid excretion; TBSAe, total secondary bile acid excretion; TAG, plasma triacylglycerides; Transit, gut transit time; Urine, total 24 h urine output; VLDL_C, plasma VLDL cholesterol.

Because the liver is central for regulation of plasma lipids, we also investigated liver function. Most of the rat studies reporting lipid-modulating effects of various forms of onion have used alloxan or streptozotozin-induced diabetic rats and report onion antihyperlipidemic and antihyperglycemic effects (*21*). In the study by El-Dermedash et al. (*22*) diabetic rats fed onion and garlic juices showed a reduction in plasma alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, and alkaline and acid phosphatases, and the authors reported that these two *Allium* vegetables can inhibit the liver and renal damage caused by alloxan-induced diabetes. Similarly to the above cited studies, which used diabetic rodents, our results revealed a decreased leakage of hepatic enzymes, except for GGT, which increased in plasma (**Table 2**). GGT is excreted from the liver to degrade glutathione and thereby increase uptake of building materials for intracellular glutathione synthesis. We found previously that glutathione synthesis in the liver was up-regulated, albeit most strongly with OR (*23*). GGT may therefore be specifically increased by the onion products due to their effects on glutathione metabolism without being a sign of general

hepatotoxicity. Overall, it could be inferred that in healthy rats these onion products did not cause any overt liver or renal damage that could affect cholesterol handling.

Rats have been used frequently as models to evaluate effects of dietary fiber sources on plasma cholesterol, and several types of fruits, vegetables, and specific fiber fractions have been found to affect plasma lipids or cholesterol (*12*, *24*, *25*). In contrast, our current results show significantly increased TAG and VLDL-C concentrations in plasma of rats fed the OR (**Figure 1**). *Hmgcr* was not significantly down-regulated as a consequence of feeding rats with either of the products (OBP, OE, or OR) (**Table 2**). In a study by Campos et al. (*26*) decreased plasma TC, TAG, and LDL-C were seen in streptozotocin diabetic rats fed onion. Later, Azuma et al. (*27*) found lower plasma TAG levels and no significant effects on cholesterol levels in diabetic rats fed an onion diet. Effects shown in other animal models such as pigs demonstrate that the consumption of onion modifies plasma lipid profiles in either healthy pigs or pigs consuming a high-fat diet but that the response was dependent on the variety of onion, feeding time, and sampling time (*28*, *29*). Our results are in agreement with these studies as far as plasma TAG, TC, and cholesterol concentrations in the LDL, and HDL fractions were unaffected by feeding with OBP and OE. Onion fractions have not been tested previously, so our observation of an increased level of cholesterol in the VLDL fraction and an increased level of plasma TAG after feeding the OR indicates that it may stimulate hepatic lipid synthesis and transport to peripheral tissues. This is often caused postprandially by fructose-rich or fatty foods (*11*). The result is unexpected here because the fructose level in the OR is much lower than in the other fractions and because the effect was observed after fasting. An altered regulation of hepatic cholesterol synthesis or loss of cholesterol metabolites in the form of bile acids could not explain the effect based on our data. We speculate that the OR components may function as a slow-release formulation for fructose, thereby keeping the animals for a longer time in a state where fructose is released. Alternatively, the OR may have an effect on other aspects of VLDL assembly or on peripheral lipoprotein lipase (i.e., TAG clearance). Such effects would then have to be counteracted by other constituents in the whole OBP. This latter possibility is corroborated by our multivariate analysis showing that OBP and OE contrast with OR, particularly due to the contrasting effects on plasma lipids. The bile acid synthetic pathway and the VLDL-C assembly/secretion pathway are known to be regulated through sterol response element-binding protein (SREBP)-dependent transcription (*30*, *31*). Therefore, additional gene expression studies would be required to assess this latter possibility.

Total and primary bile acid concentration in feces (data not shown) as well as their total 24 h excretion were not significantly increased (**Table 3**). However, there was a numerical doubling of fecal primary bile acid concentration after feeding the OE and OR, and fecal concentration of the major primary bile acid, α-muricholic acid, was even higher in rats fed the OE. It cannot be ruled out that the large variations between rats in fecal bile acid concentrations and in 24 h fecal output may have masked an effect; however, the lack of effects on total cholesterol is in agreement with the observed null result also on bile acid excretion. The rat is not a good model for agents affecting reverse cholesterol transport because the transfer of cholesterol from LDL to HDL does not proceed in a similar manner in rodents and in humans. If onion affects this mechanism, its effect has to be investigated in humans and would not be observed in the present study. The hypocholesterolemic effect of dietary fiber has been attributed to its ability to inhibit intestinal absorption of bile acids and neutral steroids, resulting in greater fecal bile acids and total steroid excretion (*32*). Several studies have reported the lipid-lowering effect of fructans and onion dietary fiber components in rats (*33*−*35*) at dose levels of around 10% in the diet, a level somewhat higher than ours. Also, onion OSCs have been reported to have hypolipidemic effects (*36*), albeit at doses far above what could be obtained through foods. Particularly, water-soluble OSCs have been reported to modulate lipid metabolism (*37*). Most of the studies conducted in this area were focused on garlic OSCs (*38*, *39*). In our study the excretion of a putative OSC metabolite was more than twice as high in the OE as in the OR fed group of animals (*6*, *7*). The contrasts in OSCs or in FOS may have affected the plasma cholesterol distribution or bile acid excretion in the current study because the OR with low contents contrasts with OBP and OR in its effects on plasma lipids as shown most clearly in the multivariate analysis. The dose levels used in studies with purified components by others were somewhat higher than achieved here with a whole food. Some of the results on cholesterol in lipoprotein fractions published by others are based on kits for measurement of HDL-C; however, we have observed that such kits do not work with rodent samples (*19*). Our chemometric method is based on modeling of lipoprotein data obtained by ultracentrifugation. It is therefore likely that previous studies may have overestimated the effects of FOS and OSCs on plasma lipoproteins in rats. Plant sterols may also affect cholesterol. Onion contains phytosterols at a level of approximately 0.1% of the dry weight (*40*). In the OBP group the daily dose of phytosterols may be calculated to be around 2 mg/day, a dose that is probably far too low for an effect. In normal rats doses of plant sterols of up to 8.1% in the diet were without effects on plasma cholesterol (*41*).

Further analyses using LC-MS-based metabolomic profiling will be carried out to search for metabolites that correlate with the biological responses observed.

In conclusion, the onion byproduct (*A. cepa* L. var. *cepa* 'Recas') or its fractions, that is, onion extract and onion residue, do not seem to strongly reduce cholesterol or affect hepatic *Hmgcr* rat gene expression. Feeding rats the onion residue increased cholesterol in the very low density lipoprotein fraction and also increased plasma triacylglycerides, indicating an up-regulation of hepatic lipid transport to peripheral tissues. Neither total bile acids nor total primary or secondary bile acids were significantly affected by feeding the onion byproduct, onion extract, or onion residue. As revealed by principal component analysis, the effects on plasma lipids differ between the onion extract and the onion residue fed groups of rats, indicating that specific bioactivities may be obtained by further refining the onion byproduct. The effects of the onion byproduct should be investigated in humans to assess the full safety of using the product as an alternative to synthetic additives, for example, as an antioxidant and antibrowning agent.

ABBREVIATIONS USED

ALAT, alanine aminotransferase; AP, alkaline phosphatase; CVD, cardiovascular disease; GGT, γ-glutamyl transferase; HDL-C, cholesterol in high-density lipoprotein; *Hmgcr*, 3-hydroxy-3-methylglutaryl-coenzyme A reductase rat gene; LDL-C, cholesterol in low-density lipoprotein; OBP, onion byproduct; OE, onion extract; OR, onion residue; OSC, organosulfur compounds; PCA, principal component analysis; TAG, triacylglycerides; TC, total cholesterol; VLDL-C, cholesterol in very low density lipoprotein.

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LITERATURE CITED

(1) Powolny, A. A.; Singh, S. V. Multitargeted prevention and therapy of cancer by diallyl trisulfide and related *Allium* vegetable-derived organosulfur compounds. *Cancer Lett*. **2008**, *269* (2), 305–314.

(2) Benavides, G. A.; Squadrito, G. L.; Mills, R. W.; Patel, H. D.; Isbell, T. S.; Patel, R. P.; Darley-Usmar, V. M.; Doeller, J. E.; Kraus, D. W. Hydrogen sulfide mediates the vasoactivity of garlic. *Proc. Natl. Acad. Sci. U.S.A*. **2007**, *104* (46), 17977–17982 .

(3) Xiao, D.; Zeng, Y.; Hahm, E. R.; Kim, Y. A.; Ramalingam, S.; Singh, S. V. Diallyl trisulfide selectively causes Bax- and Bak-mediated apoptosis in human lung cancer cells. *Environ. Mol. Mutagen*. **2009**, *50* (3), 201–212.

(4) Hertog, M. G. L.; Feskens, E. J. M.; Hollman, P. C. H.; Katan, M. B.; Kromhout, D. Dietary antioxidant flavonoids and risk of cornary heart disease: the Zutphen elderly study. *Lancet* **1993**, *342*, 1007–1011.

(5) Roldán, E.; Sánchez-Moreno, C.; de Ancos, B.; Cano, M. P. Characterisation of onion (*Allium cepa* L.) by-products as food ingredients with antioxidant and antibrowning properties. *Food Chem*. **2008**, *108* (3), 907–916.

(6) Roldan-Marin, E.; Krath, B. N.; Poulsen, M.; Binderup, M. L.; Nielsen, T. H.; Hansen, M.; Barri, T.; Langkilde, S.; Cano, M. P.; Sanchez-Moreno, C.; Dragsted, L. O. Effects of an onion by-product on bioactivity and safety markers in healthy rats. *Br. J. Nutr*. **2009**, *102* (11), 1574–1582.

(7) Winning, H.; Roldan-Marin, E.; Dragsted, L. O.; Viereck, N.; Poulsen, M.; Sanchez-Moreno, C.; Cano, M. P.; Engelsen, S. B. An exploratory NMR nutri-metabonomic investigation reveals dimethyl sulfone as a dietary biomarker for onion intake. *Analyst* **2009**, *134* (11), 2344–2351.

(8) WHO. *The World Health Report 2008 − Primary Health Care* (*Now More Than Ever*); http://www.who.int/whr/2008/en/index.html, **2008**.

(9) WHO. *Prevention of cardiovascular disease: guideline for assessment and management of cardiovascular risk*; http://www.who.int/cardiovascular_diseases/guidelines/Prevention_of_Cardiovascular_Disease/en/index.html, **2007**.

(10) WHO. *Diet and physical activity: a public health priority*; http://www.who.int/dietphysicalactivity/en/, **2004**.

(11) Hansen, M.; Baunsgaard, D.; Autrup, H.; Vogel, U.; Moller, P.; Lindecrona, R. H.; Wallin, H.; Poulsen, H. E.; Loft, S.; Dragsted, L. O. Sucrose, glucose and fructose have similar genotoxicity in the rat colon and affect the metabolism. *Food Chem. Toxicol*. **2008**, *46*, 752–760.

(12) Hara, H.; Haga, S.; Aoyama, Y.; Kiriyama, S. Short-chain fatty acids suppress cholesterol synthesis in rat liver and intestine. *J. Nutr*. **1999**, *129* (5), 942–948.

(13) Jaime, L.; Martin-Cabrejas, M. A.; Molla, E.; Lopez-Andreu, F. J.; Esteban, R. M. Effect of storage on fructan and fructooligosaccharide of onion (*Allium cepa* L.). *J. Agric. Food Chem*. **2001**, *49* (2), 982–988.

(14) Beutler, H. O. Monosaccharides and derivatives. D-Fructose. In *Methods of Enzymatic Analysis*; Bergmeyer, H. U., Ed.; Verlag Chemie: Weinheim, Germany, 1984; pp 321−327.

(15) Kunst, A.; Draeger, B.; Ziegenhorn, J. Monosaccharides and derivatives. D-Glucose. In *Methods of Enzymatic Analysis*; Bergmeyer, H. U., Ed.; Verlag Chemie: Weinheim, Germany, 1984; pp 163−172.

(16) Outlaw, W. H.; Tarczynski, M. C. Poly-, oligo-, and disaccharides. Sucrose. In *Methods of Enzymatic Analysis*; Bergmeyer, H. U., Ed.; Verlag Chemie: Weinheim, Germany, 1984; pp 96−103.

(17) Nielsen, T. H.; Skjærbæk, H. C.; Karlsen, P. Carbohydrate metabolism during fruit development in sweet peppar (*Capsicum annuum*) plants. *Physiol. Planta*. **1991**, *82*, 311–319.

(18) Blennow, A.; Bay-Smidt, A. M.; Olsen, C. E.; Wischmann, B.; Møller, B. L. The degree of starch phosphorylation is related to the chain length distribution of the neutral and phosphorylated chains of amylopectin. *Carbohydr. Res*. **1998**, *307*, 45–54.

(19) Kristensen, M.; Savorani, F.; Ravn-Haren, G.; Poulsen, M.; Markowski, J.; Larsen, F. H.; Dragsted, L. O.; Engelsen, S. B. NMR and interval PLS as reliable methods for determination of cholesterol in rodent lipoprotein fractions. *Analyst* **2009**, in press (DOI 10.1007/s11306-009-0181-3, 1−8).

(20) Waring, R. H.; Mitchell, S. C.; Fenwick, G. R. The chemical nature of the urinary odour produced by man after asparagus ingestion. *Xenobiotica* **1987**, *17* (11), 1363–1371.

(21) Islam, M. S.; Choi, H.; Loots, D. T. Effects of dietary onion (*Allium cepa* L.) in a high-fat diet streptozotocin-induced diabetes rodent model. *Ann. Nutr. Metab*. **2008**, *53* (1), 6–12.

(22) El-Demerdash, F. M.; Yousef, M. I.; El-Naga, N. I. Biochemical study on the hypoglycemic effects of onion and garlic in alloxan-induced diabetic rats. *Food Chem. Toxicol*. **2005**, *43* (1), 57–63.

(23) Roldan-Marin, E.; Krath, B. N.; Poulsen, M.; Binderup, M. L.; Nielsen, T. H.; Hansen, M.; Barri, T.; Langkilde, S.; Pilar, C. M.; Sanchez-Moreno, C.; Dragsted, L. O. Effects of an onion by-product on bioactivity and safety markers in healthy rats. *Br. J. Nutr*. **2009**, 1–9.

(24) Aprikian, O.; Levrat, M. A.; Besson, C.; Busserolles, J.; Remesy, C.; Demigne, C. Apple favourably affects parameters of cholesterol metabolism and of anti-oxidative protection in cholesterol-fed rats. *Food Chem*. **2001**, *75* (4), 445–452.

(25) Horigome, T.; Sakaguchi, E.; Kishimoto, C. Hypocholesterolaemic effect of banana (*Musa sapientum* L. var. Cavendishii) pulp in the rat fed on a cholesterol-containing diet. *Br. J. Nutr*. **1992**, *68* (1), 231–244.

(26) Campos, K. E.; Diniz, Y. S.; Cataneo, A. C.; Faine, L. A.; Alves, M. J.; Novelli, E. L. Hypoglycaemic and antioxidant effects of onion, *Allium cepa*: dietary onion addition, antioxidant activity and hypoglycaemic effects on diabetic rats. *Int. J. Food Sci. Nutr*. **2003**, *54* (3), 241–246.

(27) Azuma, K.; Minami, Y.; Ippoushi, K.; Terao, J. Lowering effects of onion intake on oxidative stress biomarkers in streptozotocin-induced diabetic rats. *J. Clin. Biochem. Nutr*. **2007**, *40* (2), 131–140.

(28) Gabler, N. K.; Osrowska, E.; Imsic, M.; Eagling, D. R.; Jois, M.; Tatham, B. G.; Dunshea, F. R. Dietary onion intake as part of a typical high fat diet improves indices of cardiovascular health using the mixed sex pig model. *Plant Foods Hum. Nutr*. **2006**, *61* (4), 179–185.

(29) Ostrowska, E.; Gabler, N. K.; Sterling, S. J.; Tatham, B. G.; Jones, R. B.; Eagling, D. R.; Jois, M.; Dunshea, F. R. Consumption of brown onions (*Allium cepa* var. Cavalier and var. Destiny) moderately modulates blood lipids, haematological and haemostatic variables in healthy pigs. *Br. J. Nutr*. **2004**, *91* (2), 211–218.

(30) Kang, S.; Davis, R. A. Cholesterol and hepatic lipoprotein assembly and secretion. *Biochim. Biophys. Acta* **2000**, *1529* (1−3), 223–230.

(31) Ness, G. C.; Chambers, C. M. Feedback and hormonal regulation of hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase: the concept of cholesterol buffering capacity. *Proc. Soc. Exp. Biol. Med*. **2000**, *224* (1), 8–19.

(32) Han, K. H.; Iijuka, M.; Shimada, K.; Sekikawa, M.; Kuramochi, K.; Ohba, K.; Ruvini, L.; Chiji, H.; Fukushima, M. Adzuki resistant starch lowered serum cholesterol and hepatic 3-hydroxy-3-methylglutaryl-CoA mRNA levels and increased hepatic LDL-receptor and cholesterol 7α-hydroxylase mRNA levels in rats fed a cholesterol diet. *Br. J. Nutr*. **2005**, *94* (6), 902–908.

(33) Busserolles, J.; Gueux, E.; Rock, E.; Demigne, C.; Mazur, A.; Rayssiguier, Y. Oligofructose protects against the hypertriglyceridemic and pro-oxidative effects of a high fructose diet in rats. *J. Nutr*. **2003**, *133* (6), 1903–1908.

(34) Delzenne, N. M.; Kok, N. Effects of fructans-type prebiotics on lipid metabolism. *Am. J. Clin. Nutr*. **2001**, *73* (2 Suppl), 456S-458S.

(35) Nishimura, N.; Taniguchi, Y.; Kiriyama, S. Plasma cholesterol-lowering effect on rats of dietary fiber extracted from immature plants. *Biosci., Biotechnol., Biochem*. **2000**, *64* (12), 2543–2551.

(36) Kumari, K.; Augusti, K. T. Lipid lowering effect of *S*-methyl cysteine sulfoxide from *Allium cepa* Linn in high cholesterol diet fed rats. *J. Ethnopharmacol*. **2007**, *109* (3), 367–371.

(37) Lin, C. C.; Yin, M. C. Effects of cysteine-containing compounds on biosynthesis of triacylglycerol and cholesterol and anti-oxidative protection in liver from mice consuming a high-fat diet. *Br. J. Nutr.* **2008**, *99* (1), 37–43.

(38) Liu, L.; Yeh, Y. Y. Water-soluble organosulfur compounds of garlic inhibit fatty acid and triglyceride syntheses in cultured rat hepatocytes. *Lipids* **2001**, *36* (4), 395–400.

(39) Liu, L.; Yeh, Y. Y. *S*-Alk(en)yl cysteines of garlic inhibit cholesterol synthesis by deactivating HMG-CoA reductase in cultured rat hepatocytes. *J. Nutr.* **2002**, *132* (6), 1129–1134.

(40) Piironen, V.; Toivo, J.; Puupponen-Pimia, R.; Lampi, A.-M. Plant sterols in vegetables, fruits and berries. *J. Sci. Food Agric.* **2003**, *83*, 330–337.

(41) Hepburn, P. A.; Horner, S. A.; Smith, M. Safety evaluation of phytosterol esters. Part 2. Subchronic 90-day oral toxicity study on phytosterol esters − a novel functional food. *Food Chem. Toxicol.* **1999**, *37* (5), 521–532.

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