DOI 10.1002/mnfr.201100231 *Mol. Nutr. Food Res.* 2011, *55*, S290–S293

FOOD & FUNCTION

Rye bran alkylresorcinols suppress adipocyte lipolysis and hormone-sensitive lipase activity

Ulrika Andersson[1], Estera S. Dey[2], Cecilia Holm[1] and Eva Degerman[1]

[1] Department of Experimental Medical Science, Lund University, Lund, Sweden
[2] Department of Pure and Applied Biochemistry, Lund University, Lund, Sweden

The effects of alkylresorcinols (ARs) isolated from rye bran on adipocyte lipolysis, hormone-sensitive lipase activity and phosphorylation and on phosphorylation of protein kinase A substrates were studied. Preincubation with ARs for 18 h suppressed catecholamine-stimulated lipolysis in 3T3-L1 adipocytes. Furthermore, phosphorylation of hormone-sensitive lipase (HSL), a key lipase responsible for stimulated lipolysis, and phosphorylation of protein kinase A substrates, were diminished after preincubation with ARs, whereas HSL protein expression was unaltered. ARs were also shown to inhibit HSL activity in an in vitro assay.

Received: April 4, 2011
Revised: June 23, 2011
Accepted: July 9, 2011

Keywords:
Adipose tissue / Alkylresorcinols / Lipolysis / Type 2 diabetes / Whole grain

Intake of whole grain products is associated with a decreased risk of type 2 diabetes [1–3] and other metabolic diseases. Alkylresorcinols (ARs) are a group of phenolic lipids present in the outer layer of mainly whole grain rye and whole grain wheat (> 500 μg/g). They have an odd-numbered alkyl chain attached to position 5 of the 1,3-dihydroxybenzene ring [4]. The length of the alkyl side chain of ARs varies from 13 to 27 carbon atoms [5, 6]. The side chain is usually saturated but unsaturated and oxygenated chain analogues have also been reported [7]. Since ARs occur only in the outer layer of the grain, it has been proposed that ARs can serve as a biomarker for whole grain rye and wheat intake [8, 9]. The content of ARs in whole grain products is relatively high (e.g. whole grain rye crisp bread 886–1007 μg/g; whole grain rye bread 380–707 μg/g) [9]. ARs are absorbed in the small intestine [10, 11], transported via the lymphatic system and carried in association with erythrocyte membranes and lipoproteins in the blood [10, 12, 13]. In vitro studies have shown that ARs incorporated in biological membranes possess the ability to alter the properties and function of membranes [14], including the activity of membrane-bound enzymes, thus suggesting a role for ARs in cell metabolism [7]. Ross et al. demonstrated the presence of ARs in adipose tissue of rats fed a diet rich in purified ARs [15] and it was recently shown that intake of whole grain bread correlates with the content of ARs in human adipose tissue [16].

Dysregulated lipid metabolism plays a central role in the development of type 2 diabetes and elevated levels of free fatty acids (FFA) in plasma together with ectopic fat deposition are associated with the development of insulin resistance [17]. Lipolysis is the process leading to release of FFA from stored triacylglycerol (TAG) into the blood stream, where they are transported to other tissues to be used as energy substrate. Hormone-sensitive lipase (HSL), together with other lipases and perilipin, a fat droplet associated protein, are responsible for mobilizing FFA from the adipose tissue [18]. HSL is phosphorylated and activated by protein kinase A (PKA) in response to a catecholamine-induced rise in intracellular cAMP levels [19, 20].

In view of the importance of adipose tissue in the development of insulin resistance and the fact that ARs have been found to accumulate in adipose tissue, we here examined the effects of rye bran ARs on lipolysis and HSL activity using 3T3-L1 adipocytes as experimental model. The ARs were extracted and pre-purified from rye bran (''Amilo'', Sweden, 2008) and the single homologues were isolated by preparative high-pressure liquid chromatography [21]. In the biological tests the highly purified homologues

Correspondence: Dr. Ulrika Andersson, Klinikgatan 28, BMC C11, SE-22184 Lund, Sweden
E-mail: ulrika.andersson@med.lu.se
Fax: +46-46-2224022

Abbreviations: ARs, alkylresorcinols; **ATGL,** adipose triglyceride lipase; **FFA,** free fatty acids; **HSL,** hormone-sensitive lipase; **PKA,** protein kinase A; **TAG,** triacylglycerols

C17 and C19 were used. The homologues were eluted from the C8 reversed-phase preparative column using ethanol/water gradient after 140 and 167 min, respectively. For all analyses, ARs were diluted in 99.5% ethanol and sonicated for 3×10 s. Ethanol was added to all assays at a final concentration of 1% v/v. In Supporting Information, details regarding the culturing and differentiation of 3T3-L1 adipocytes, measurements of lipolysis, measurements of HSL activity, SDS page and Western blot analysis and statistical analyses are provided.

Preincubation with ARs for 18 h with the C17 homologue suppressed lipolysis induced by 10 and 100 nM isoproterenol (Fig. 1), whereas preincubation for 6 h had no effect (data not shown). A tendency towards suppression of basal lipolysis after preincubation with ARs was also observed, but it was not statistically significant. To examine the dose dependency of the effect of ARs, 3T3-L1 adipocytes were incubated with three different concentrations of ARs (0.34, 3.4 and 34 μM). Isoproterenol-stimulated lipolysis was significantly suppressed using 34 μM ARs, whereas the lower concentrations did not appear to have any effect (data not shown). Similar results were obtained using the C19 homologue (data not shown). The effects of ARs on isoproterenol-induced phosphorylations were studied using antibodies against consensus sequences for phosphorylation by PKA. Preincubation of 3T3-L1 adipocytes with ARs resulted in reduced phosphorylation of some of the phosphorylation targets (Fig. 2A, upper panel). The expression level of the PKA regulatory subunit did not differ significantly during the different conditions (Fig. 2A, lower panel). HSL, a key enzyme in stimulated lipolysis, is a target for PKA. Preincubation of 3T3-L1 adipocytes with the C17 homologue resulted in a significantly reduced phosphorylation at Ser563, a site known to be phosphorylated by PKA. The expression level of HSL was not affected by AR treatment (Fig. 2B). No significant effect on the phosphorylation of HSL at Ser563 was observed after preincubation with ARs for 6 h (data not shown). Preincubation with ARs for 18 h did not result in altered expression of total perilipin or in altered ability of isoproterenol to induce a mobility shift of perilipin, an indirect measurement of perilipin phosphorylation (Supporting Information, Fig. 1).

We also investigated the ability of ARs to directly inhibit purified HSL in a cell-free system. Preincubation of HSL with 38 μM ARs for 30 min resulted in decreased lipase activity (Fig. 3). A trend towards a dose-dependent inhibition was observed, but the inhibition exerted by the two lower concentrations of ARs did not attain statistical

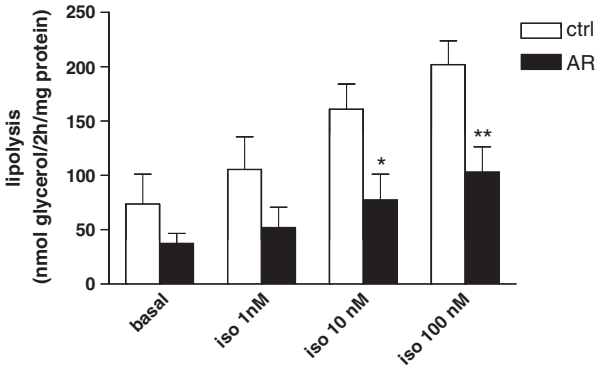

Figure 1. The effect of 18 h of preincubation with the AR homologue C17 (34 μM) on basal and stimulated lipolysis compared to control (ctrl) using the indicated concentrations of isoproterenol (iso) in 3T3-L1 adipocytes. Data are mean ± SEM, $n = 5$. $^{*}p < 0.05$, $^{**}p < 0.01$.

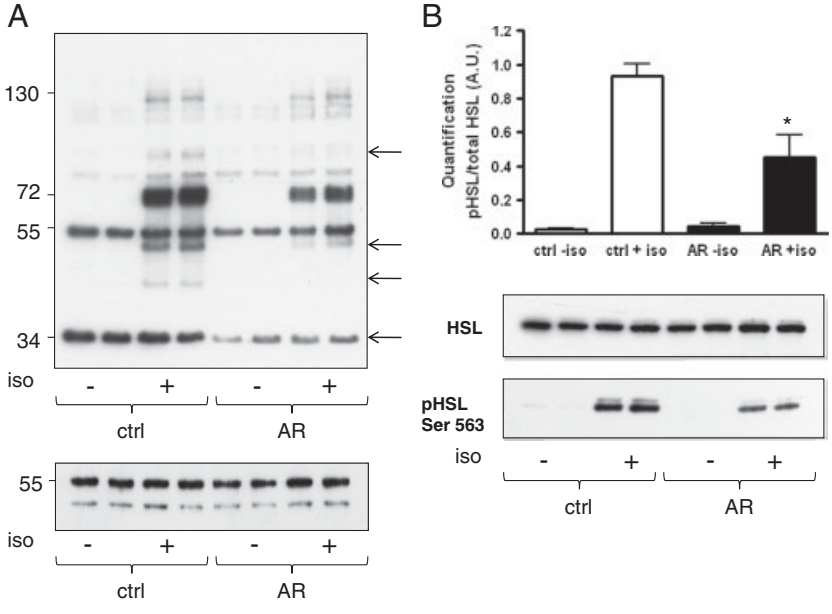

Figure 2. After 18 hours of preincubation of 3T3-L1 adipocyte with 34 μM of the AR homologue C17 (AR) or without (ctrl) the cells were incubated with (+) or without (−) 100 nM of isoproterenol (iso) for 2 hours. Cell homogenates were analyzed by Western blot analysis using antibodies against consensus sequences for phosphorylation by PKA (RRXS(P)/T(P)) (A, upper panel) and antibodies against the regulatory subunit of PKA (A, lower panel) as well as antibodies against total HSL and pHSL Ser563 (B). The samples are loaded in duplicates on the gel. The arrows indicate PKA targets with reduced phosphorylation after AR treatment. HSL and pHSL were quantified and expressed as mean pHSL/HSL ± SEM from three separate experiments. Representative blots are shown. $^{*}p < 0.05$.

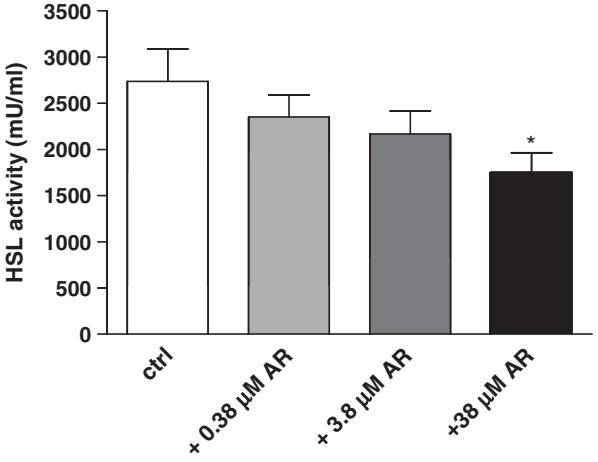

Figure 3. HSL activity after preincubation with the indicated AR concentrations for 30 min and then assayed using tri [^{3}H]oleoylglycerol as substrate. The homologue C19 was used in these experiments. One unit of enzyme activity is equivalent to 1 μmol of fatty acids released/min at 37°C. Data are mean ± SEM, $n = 6$. $^*p < 0.05$

significance. HSL activity assays were performed using the C19 homologue.

In summary, we show that ARs inhibit catecholamine-stimulated lipolysis in 3T3-L1 adipocytes as well as the activity of HSL, the latter demonstrated both by an in vitro assay and by diminished phosphorylation. The diminished phosphorylation of HSL is presumably a result of the reduced PKA activity. The concentration needed to obtain effects in this study, 34 μM, is higher than the plasma levels estimated after intake of whole grain in humans (0.2–0.5 μM) [22], however, in the human studies the concentration of ARs adjacent to the adipocytes is not known. Also, in a physiological context, the transport and uptake of ARs could be facilitated by mechanisms not available in an intact cell model system.

Under basal conditions HSL is almost exclusively found in the cytosol of the adipocyte. Upon phosphorylation and activation by PKA, HSL translocates from the cytosol to the surface of the lipid droplet where it hydrolyses stored TAG [18]. ARs have the ability to incorporate into cellular membranes due to their amphiphilic properties and they have been shown to accumulate in adipose tissue in both rats and humans [15, 16]. It is also known that ARs have the ability to directly interact with proteins by binding to a hydrophobic region [7]. Based upon this, it can be hypothesized that ARs have the capacity to diffuse across the plasma membrane and to associate with the lipid droplet and interact with HSL. However, additional studies are needed to prove the ability of ARs to cross the adipocyte membrane. In future studies it would also be of interest to investigate whether the inhibitory effect of ARs applies to other lipases, including adipose triglyceride lipase (ATGL), the other major lipase in adipose tissue lipolysis [18]. ATGL has been shown to be involved in basal as well as stimulated lipolysis acting together with CGI-58/ABHD5 [18]. With regard to direct effects of ARs on metabolic enzymes, it has previously been shown that ARs isolated from wheat and rye bran inhibit in vitro activity of glycerol-3-phosphate dehydrogenase (GPDH), a key enzyme in TAG synthesis, and TAG accumulation in 3T3-L1 preadipocytes [23, 24]. However, the fact that these experiments were performed in differentiating fibroblasts and not in fully mature adipocytes makes it difficult to assess whether the effect of ARs is on lipogenesis per se or on differentiation in general (adipogenesis).

In contrast to the effect of ARs on HSL activity, which most likely is a direct effect on HSL, the inhibitory effect of ARs on catecholamine-stimulated phosphorylation of HSL and lipolysis in 3T3-L1 adipocytes presumably reflects the observed reduction in PKA signaling. More studies are needed to elucidate the mechanisms underlying the reduction in PKA signaling, but it could be speculated that AR treatment exert its effects at the level of the plasma membrane/lipid rafts where the β-adrenergic receptor is localized (or at the level of downstream targets).

Cereals constitute a major source of dietary carbohydrates in western countries. However, cereals are often consumed as refined products thereby lacking ARs and other components associated with the bran. Interest in the potential health benefits from whole grain cereals has increased in the past years. ARs are one of several bioactive components in whole grain that could play a role in the protective effect of whole grain regarding diabetes risk. Our results suggest that a constant high intake of ARs, in the format of for example whole grain rye, could lead to a reduced lipolysis in vivo and hence lower levels of circulating FFA.

This study was supported by the Lund University Antidiabetic Food Center, a VINNOVA VINN Excellence Center and the FuncFood PhD program at Lund University. The authors thank Eva Ohlson, Ann-Kristin Holmén-Pålbrink, Birgitta Danielsson and Sara Larsson for excellent technical assistance.

The authors have declared no conflict of interest.

References

[1] de Munter, J. S., Hu, F. B., Spiegelman, D., Franz, M., van Dam, R. M., Whole grain, bran, and germ intake and risk of type 2 diabetes: a prospective cohort study and systematic review. *PLoS Med.* 2007, *4*, e261.

[2] Montonen, J., Knekt, P., Jarvinen, R., Aromaa, A., Reunanen, A., Whole-grain and fiber intake and the incidence of type 2 diabetes. *Am. J. Clin. Nutr.* 2003, *77*, 622–629.

[3] Slavin, J., Why whole grains are protective: biological mechanisms. *Proc. Nutr. Soc.* 2003, *62*, 129–134.

[4] Ross, A. B., Shepherd, M. J., Schupphaus, M., Sinclair, V. et al., Alkylresorcinols in cereals and cereal products. *J. Agric. Food Chem.* 2003, *51*, 4111–4118.

[5] Knodler, M., Kaiser, A., Carle, R., Schieber, A. et al., Profiling of Alk(en)ylresorcinols in cereals by HPLC-DAD-APcI-MSn. *Anal. Bioanal. Chem.* 2008, *391*, 221–228.

[6] Kozubek, A., Cereal grain resorcinolic lipids: mono and dienic homologues are present in rye grains. *Chem. Phys. Lipids* 1995, *78*, 29–35.

[7] Kozubek, A., Tyman, J. H., Resorcinolic lipids, the natural non-isoprenoid phenolic amphiphiles and their biological activity. *Chem. Rev.* 1999, *99*, 1–26.

[8] Landberg, R., Kamal-Eldin, A., Andersson, A., Vessby, B., Aman, P., Alkylresorcinols as biomarkers of whole-grain wheat and rye intake: plasma concentration and intake estimated from dietary records. *Am. J. Clin. Nutr.* 2008, *87*, 832–838.

[9] Ross, A. B., Kamal-Eldin, A., Aman, P., Dietary alkylresorcinols: absorption, bioactivities, and possible use as biomarkers of whole-grain wheat- and rye-rich foods. *Nutr. Rev.* 2004, *62*, 81–95.

[10] Ross, A. B., Kamal-Eldin, A., Lundin, E. A., Zhang, J. X. et al., Cereal alkylresorcinols are absorbed by humans. *J. Nutr.* 2003, *133*, 2222–2224.

[11] Ross, A. B., Shepherd, M. J., Bach Knudsen, K. E., Glitso, L. V. et al., Absorption of dietary alkylresorcinols in ileal-cannulated pigs and rats. *Br. J. Nutr.* 2003, *90*, 787–794.

[12] Linko-Parvinen, A. M., Landberg, R., Tikkanen, M. J., Adlercreutz, H., Penalvo, J. L., Alkylresorcinols from whole-grain wheat and rye are transported in human plasma lipoproteins. *J. Nutr.* 2007, *137*, 1137–1142.

[13] Linko, A. M., Adlercreutz, H., Whole-grain rye and wheat alkylresorcinols are incorporated into human erythrocyte membranes. *Br. J. Nutr.* 2005, *93*, 11–13.

[14] Kozubek, A., The effect of 5-(*n*-alk(en)yl)resorcinols on membranes. I. Characterization of the permeability increase induced by 5-(*n*-heptadecenyl)resorcinol. *Acta Biochim. Pol.* 1987, *34*, 357–367.

[15] Ross, A. B., Chen, Y., Frank, J., Swanson, J. E. et al., Cereal alkylresorcinols elevate gamma-tocopherol levels in rats and inhibit gamma-tocopherol metabolism in vitro. *J. Nutr.* 2004, *134*, 506–510.

[16] Jansson, E., Landberg, R., Kamal-Eldin, A., Wolk, A. et al., Presence of alkylresorcinols, potential whole grain biomarkers, in human adipose tissue. *Br. J. Nutr.* 2010, *104*, 633–636.

[17] Samuel, V. T., Petersen, K. F., Shulman, G. I., Lipid-induced insulin resistance: unravelling the mechanism. *Lancet* 2010, *375*, 2267–2277.

[18] Schweiger, M., Schreiber, R., Haemmerle, G., Lass, A. et al., Adipose triglyceride lipase and hormone-sensitive lipase are the major enzymes in adipose tissue triacylglycerol catabolism. *J. Biol. Chem.* 2006, *281*, 40236–40241.

[19] Fredrikson, G., Stralfors, P., Nilsson, N. O., Belfrage, P., Hormone-sensitive lipase of rat adipose tissue. Purification and some properties. *J. Biol. Chem.* 1981, *256*, 6311–6320.

[20] Stralfors, P., Belfrage, P., Phosphorylation of hormone-sensitive lipase by cyclic AMP-dependent protein kinase. *J. Biol. Chem.* 1983, *258*, 15146–15152.

[21] Dey, E. S., Mikhailopulo, K., A food grade approach for the isolation of major alkylresorcinols (ARs) from rye bran applying tailored supercritical carbon dioxide ($scCO_2$) extraction combined with HPLC. *J. Supercrit. Fluid.* 2009, *51*, 167–173.

[22] Landberg, R., Aman, P., Friberg, LE., Vessby, B., Adlercreutz, H., Kamal-Eldin, A., Dose response of whole-grain biomarkers: alkylresorcinols in human plasma and their metabolites in urine in relation to intake. *Am. J. Clin. Nutr.* 2009, *89*, 290–296.

[23] Tsuge, N., Mizokami, M., Imai, S., Shimazu, A., Seto, H., Adipostatins A and B, new inhibitors of glycerol-3-phosphate dehydrogenase. *J. Antibiot. (Tokyo)* 1992, *45*, 886–891.

[24] Rejman, J., Kozubek, A., Inhibitory effect of natural phenolic lipids upon NAD-dependent dehydrogenases and on triglyceride accumulation in 3T3-L1 cells in culture. *J. Agric. Food Chem.* 2004, *52*, 246–250.

