adjustment for multiple confounders, regular consumption of dark chocolate associated with lower levels of serum CRP concentration ($P = 0.038$). A J-shaped dose–response relationship was observed with those consuming ≤ 20 g of dark chocolate per day having significantly lower serum CRP concentrations than either non-consumers or those consuming ≥ 20 g chocolate per day.

In a randomised, crossover trial of 20 obese but healthy subjects given a control beverage or one of three cocoa beverages containing 180–900 mg flavanol daily for five days, cocoa consumption dose-dependently decreased the circulating levels of CRP by 16% and 8-isoprostane by 5.7% (Stote *et al.*, 2012).

Several studies have failed to find an anti-inflammatory effect in unhealthy patients. In a study of 20 hypertensive patients, consumption of a flavanol-rich cocoa drink (900 mg flavanols/day) for two weeks failed to decrease the plasma levels of tumour necrosis factor α, interleukin (IL)-1β or IL-6, although the average brachial artery diameter was increased compared to placebo group (Muniyappa *et al.*, 2008). Monagas *et al.* (2009) reported the effect of a four week randomised cross-over trial of 40 g cocoa powder in skimmed milk daily versus skimmed milk in 42 patients at high risk of cardiovascular disease. Plasma levels of monocyte chemotactic protein (MCP)-1, IL-6 or CRP were unchanged; however, serum concentrations of P-selectin and intracellular adhesion molecule-1 were lower after the cocoa powder intervention. Thus, there is some evidence that cocoa and cocoa-rich foods might reduce low-grade systemic inflammation but additional human intervention studies are needed.

Dietary supplementation with cocoa and cocoa polyphenols has been shown to reduce inflammation in a number of animal models. For example, we have reported that dietary supplementation with cocoa powder reduced plasma levels of MCP-1 and IL-6 in high fat-fed obese mice (Gu *et al.*, 2014a). Moreover, supplementation reduced the expression of inflammatory mediators by adipose-associated macrophages (Gu *et al.*, 2014b). These cells play a key role in the development of insulin resistance and type II diabetes.

22.9 Neuroprotective and cognitive effects

A somewhat limited number of studies have examined the neuroprotective and cognitive function effects of cocoa and chocolate. Caffeine and, to a lesser extent, theobromine, are known to have central nervous system (CNS) stimulatory effects. Consumption of these compounds is associated with increased awareness and heightened focus. Recent studies have also focused on the polyphenols present in cocoa.

Population-based studies on the neuroprotective effects of chocolate are limited, but the results tend to be positive. For example, a cross-sectional study of elderly Norwegians found a positive association between cognitive performance and intake of flavonoids from chocolate, tea and wine (Nurk *et al.*, 2009).

A prospective cohort study of 1650 subjects aged 65 years or older found that, after a 10-year follow-up, subjects in the lowest quartile of chocolate consumption showed a loss of cognitive performance (Mini-Mental State Examination) that was twice as large as subjects in the highest quartile (Letenneur *et al.*, 2007).

Animal model studies tend to support the neuroprotective effects suggested by population studies. Oral administration of high flavanol-containing cocoa mitigated age-related declines in maze performance in rats. Interestingly, treatment also extended lifespan (Bisson *et al.*, 2008). In a study of Tg mice, which overexpress human amyloid precursor protein and develop amyloid plaques, supplementation with a diet enriched with cocoa and dried fruits, reduced plaque formation in the brain and reduced amyloid-associated cognitive decline (Fernandez-Fernandez *et al.*, 2012).

The methylxanthines present in cocoa, especially the caffeine, likely contribute to the neuroprotective effects of chocolate and cocoa. Both observational studies as well as human intervention studies have suggested neuroprotective and psychostimulatory effects of caffeine. For example, a population-based cohort study of more than 7000 French subjects aged 65 years and older found an inverse association between caffeine consumption and loss of verbal recall and visuospatial memory (Ritchie *et al.*, 2007). Interestingly, the effects were confined to women, and there was no association between caffeine consumption and risk of dementia. The results may suggest that caffeine can mitigate the symptoms of cognitive decline, but not the underlying cause. Human intervention studies have shown that caffeine has been shown to affect cognitive performance even at doses as low as 12.5 mg (Smit and Rogers 2000). This finding is significant, because the effective doses are similar to those found in chocolate (20–43 mg/100 g).

Chocolate and cocoa consumption have been shown in some studies to improve memory and mood. In mice, dietary treatment with pure EC increased angiogenesis in the hippocampus, the area of the brain associated with memory and learning (van Praag *et al.*, 2007). This effect would be expected to result in enhanced blood flow to the hippocampus. Consistent with this finding, a small study in 16 healthy subjects found that ingestion of a high cocoa flavanol beverage (450 mg dose) resulted in increased cerebral blood flow in a time-dependent manner (Francis *et al.*, 2006). The kinetics of this increased blood flow correlated well with the pharmacokinetics of EC in human subjects (Lee *et al.*, 2002). Studies to examine the effect of cocoa and chocolate on cognition are limited and have mixed results (Sokolov *et al.*, 2013). Additional studies are needed to determine if consumption of chocolate and cocoa-containing foods really do improve memory.

Studies in both animals and human subjects have demonstrated effects of chocolate and cocoa consumption on mood and mood-related biomarkers. For example, treatment of rats with cocoa for two weeks improved performance in the elevated T-maze test, an indication of decreased anxiety (Yamada *et al.*, 2009). Longer-term tests, showed that cocoa treatment increased brain concentrations