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Prospects for Quantitative fMRI: Investigating the Effects of Caffeine on Baseline Oxygen Metabolism and the Response to a Visual Stimulus in Humans

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Abstract

Functional magnetic resonance imaging (fMRI) provides an indirect reflection of neural activity change in the working brain through detection of blood oxygenation level dependent (BOLD) signal changes. Although widely used to map patterns of brain activation, fMRI has not yet met its potential for clinical and pharmacological studies due to difficulties in quantitatively interpreting the BOLD signal. This difficulty is due to the BOLD response being strongly modulated by two physiological factors in addition to the level of neural activity: the amount of deoxyhemoglobin present in the baseline state and the coupling ratio, n , of evoked changes in blood flow and oxygen metabolism. In this study, we used a quantitative fMRI approach with dual measurement of blood flow and BOLD responses to overcome these limitations and show that these two sources of modulation work in opposite directions following caffeine administration in healthy human subjects. A strong 27% reduction in baseline blood flow and a 22% increase in baseline oxygen metabolism after caffeine consumption led to a decrease in baseline blood oxygenation and was expected to increase the subsequent BOLD response to the visual stimulus. Opposing this, caffeine reduced n through a strong 61% increase in the evoked oxygen metabolism response to the visual stimulus. The combined effect was that BOLD responses pre- and post-caffeine were similar despite large underlying physiological changes, indicating that the magnitude of the BOLD response alone should not be interpreted as a direct measure of underlying neurophysiological changes. Instead, a quantitative methodology based on dual-echo measurement of blood flow and BOLD responses is a promising tool for applying fMRI to disease and drug studies in which both baseline conditions and the coupling of blood flow and oxygen metabolism responses to a stimulus may be altered.

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Author Contributions

J.E.P. and R.B.B. designed the experiments. J.E.P. conducted fMRI experiments. V.E.M.G. performed data analysis. V.E.M.G. and R.B.B. prepared the figures and wrote the paper. R.B.B. supervised all aspects of the work.

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Keywords

Blood oxygenation level dependent; cerebral blood flow; cerebral metabolic rate of oxygen; caffeine; functional MRI; arterial spin labeling

1. Introduction

Functional magnetic resonance imaging (fMRI) is a powerful tool to localize metabolic activity and blood flow changes resulting from brain activity. It is widely used for mapping spatial and temporal patterns of neural activity in fields as diverse as neuroscience, psychology, and economics, but fMRI has not lived up to its promise in the field of medicine. Although there are a number of potential clinical applications of fMRI, they have thus far been limited because of the complexity of the BOLD response. The BOLD signal results from changes in local deoxyhemoglobin content (Ogawa et al., 1993), which depends on the relative changes in cerebral blood flow (CBF) and the cerebral metabolic rate of oxygen (CMRO₂). The primary physiological phenomenon underlying the BOLD response is that CBF increases much more than CMRO₂ with neural stimulation, reducing the local deoxyhemoglobin content and increasing the MR signal.

The magnitude of the BOLD signal depends strongly on two additional factors: (1) the exact balance of the changes in CBF and CMRO₂, which have opposite effects on the change in blood oxygenation, and (2) how much deoxyhemoglobin is present in the baseline state (Buxton, 2010). This complexity of the signal leads to a fundamental problem in interpreting the magnitude of the BOLD response: for example, if the magnitude of the BOLD response in the hippocampus to a standard memory task is altered in disease or after administration of a drug, how should this be interpreted? Such a finding could mean that the neural response to the stimulus is altered, but it could also be due to disease- or drug-related chronic changes in the baseline state (Ances et al., 2009; Brown et al., 2003; Fleisher et al., 2009) or altered coupling of the CBF and CMRO₂ responses to the stimulus (Ances et al., 2008). The inability of BOLD-fMRI to disentangle these possible effects underlying changes in the BOLD response amplitude is the primary limitation to a broader application of fMRI as a quantitative probe of brain function.

Adding quantitative measurements of CBF based on arterial spin labeling (ASL) methods can help to untangle these confounding effects in three ways. First, ASL techniques provide information on CBF in the baseline state and on how CBF changes with activation. Second, the ASL measurement is more stable in the face of scanner drift, so that it is possible to measure changes in activity between two states widely separated in time (Borogovac et al., 2010; Wang et al., 2003). Third, while the complex sensitivity of the BOLD signal creates difficulty in interpretation, it also offers the possibility of calculating changes in CMRO₂ when BOLD and CBF measurements are combined. The calibrated-BOLD approach proposed by Davis et al. (Davis et al., 1998) utilizes this additional measurement of CBF to estimate the CMRO₂ change during brain activation, and offers the potential to broaden fMRI from a mapping tool into a true probe of brain function in health and disease.

In this paper, we show how a quantitative fMRI approach can be used to more fully assess how a drug alters both baseline CBF and CMRO₂ and the CBF and CMRO₂ responses to a standard stimulus. A quantitative methodology requires simultaneous measurements of CBF and BOLD responses and improved temporal stability of the BOLD response. The dual-echo spiral ASL pulse sequence (Wong et al., 1998) addresses these requirements by providing both CBF and BOLD time series (Fig. 1). In addition, quantification and comparison of BOLD signals widely separated in time is made possible through measurement of the

absolute transverse relaxation rate (R_2^*), which is the physical parameter underlying the BOLD effect. Using this methodology we examined the effect of caffeine as a model drug affecting both baseline neurophysiology and the neural response to a simple visual stimulus.

Caffeine, part of the methylxanthine family of chemicals, acts as a non-selective antagonist of adenosine receptors, especially types A_1 , A_{2A} , and A_{2B} (Fredholm et al., 1999; Pelligrino et al., 2010). Typically adenosine acts to inhibit release of excitatory neurotransmitters at A_1 receptors. By blocking these receptors the inhibitory activity is lifted, thereby increasing neuronal firing rate (Dunwiddie and Masino, 2001; Fredholm et al., 1999). In addition, adenosine acts as a vasodilator through A_{2A} and A_{2B} receptors located on blood vessels, and by blocking these receptors caffeine decreases CBF (Kusano et al., 2010; Pelligrino et al., 2010). This dual action of caffeine on both neural activity and blood flow through different regulatory mechanisms leads to the physiological uncoupling of CBF and $CMRO_2$, which makes caffeine an ideal drug for studying how changes in both CBF and $CMRO_2$ affect the BOLD response.

Several studies have found that caffeine reduces baseline CBF (Cameron et al., 1990a; Field et al., 2003; Mathew and Wilson, 1985), while studies on how caffeine affects the BOLD response to a stimulus have yielded conflicting results (Laurienti et al., 2003; Liao et al., 2008; Mulderink et al., 2002). In comparison, there is relatively little information available on how caffeine affects $CMRO_2$. A recent study combining CBF and BOLD response measurements found that the coupling ratio of the CBF and $CMRO_2$ stimulus responses ($n=\delta CBF/\delta CMRO_2$) decreased after caffeine administration (Chen and Parrish, 2009b), and another study from the same group found different dose-dependent effects on the BOLD and CBF responses (Chen and Parrish, 2009a). Yet, we were unable to find a study specifically measuring the combined effect of caffeine on baseline $CMRO_2$ and the evoked $CMRO_2$ response to a stimulus. This would provide a greater understanding of how caffeine affects neural metabolism. One study in preterm infants using indirect calorimetry found that caffeine increased total body oxygen consumption (Bauer et al., 2001) while another study in preterm infants demonstrated reduced cerebral oxygenation after administration of caffeine (Tracy et al., 2010). How these studies translate to cerebral oxygen metabolism in adults is unknown.

For the current study, an analysis of the baseline effects of caffeine on CBF and $CMRO_2$ in these data was previously reported (Perthen et al., 2008), while here we present an integrated analysis of the modulations of both the baseline state and the evoked responses incorporating a new model for the BOLD response. Our primary findings were: 1) that caffeine produced a significant uncoupling in the baseline state, reducing CBF while increasing $CMRO_2$; and 2) that caffeine significantly increased the absolute $CMRO_2$ response to the visual stimulus. These effects are consistent with the inhibition of the dual action of adenosine, which lowers CBF while increasing neural excitability. This raises baseline $CMRO_2$ and increases the $CMRO_2$ response to the stimulus. However, despite these large physiological changes, the standard BOLD response to the stimulus was unaltered by caffeine, emphasizing the limitations of BOLD-fMRI alone to detect the underlying physiological changes.

2. Methods

The study was performed on ten healthy adults reporting moderate daily caffeine intake (100 – 250 mg) who had abstained from caffeine consumption for at least 12 hours prior to study participation. The institutional review board at the University of California San Diego approved the study, and written informed consent was obtained from all participants. Two functional scans were performed in each of the pre- and post-caffeine states. The functional

scans consisted of four blocks of a black and white checkerboard flashing at 8 Hz for 20 s alternating with 60 s of a small stationary white square on a gray background. After the pre-caffeine functional scans, two 7-min (3-min of CO₂) hypercapnia scans were performed for calibration of the CMRO₂ calculations (see Supplementary Data, Table S1). Subjects were then removed from the scanner and given a 200 mg caffeine tablet. After 30 min, subjects were placed back into the scanner and the scan protocol was repeated without the hypercapnia scan.

Using a spiral dual-echo ASL PICORE QUIPSS II (Wong et al., 1998) pulse sequence, we simultaneously measured the CBF and BOLD responses to a strong visual stimulus before and after administration of caffeine. ASL was used to directly measure CBF by magnetically labeling arterial water with an applied RF pulse. The tagged water was allowed to flow into the slice of interest followed by tagged image (T) acquisition. A control image (C) was acquired by repeating this sequence without magnetically tagging the water. Signal acquisition occurred at two echo times (TE) every 2.5 s (TE₁=2.9 ms and TE₂=24 ms). Surround average and difference time courses were computed using the weighting shown in Figure 1.

Statistical analysis of the functional data was performed using a general linear model (GLM) approach similar to the method used in Perthen et al. (Perthen et al., 2008). An active visual cortex ROI was defined as voxels exhibiting CBF activation in the first echo difference data of the concatenated functional scans. The desired ROI size was set to 250±25 voxels for both the pre- and post-caffeine runs. The minimum acceptable correlation coefficient was decreased until this ROI size was established with the additional requirement that voxels were included in the ROI only if there were two neighboring voxels that were also above the correlation coefficient cutoff. The intersection of the pre-caffeine ROI and post-caffeine ROI was taken for each subject resulting in variable size of the CBF ROI between 100 and 167. The minimum CBF correlation coefficient varied between subjects from 0.11 to 0.28. Analysis was limited to voxels exhibiting a minimum signal to noise ratio of 100 and minimum CBF signal of 30% of the mean baseline CBF to preferentially increase the likelihood of gray matter over white matter inclusion in the ROI. For summary statistics, the baseline was averaged over the 10 s prior to the start of the stimulus and the stimulus response was averaged over the last 10 s of the stimulus. To test whether our method of ROI determination biased our results, we also analyzed our data using a combined BOLD/CBF ROI.

The surround average signal (A) of the tag and control images was calculated for each subject at the two echo times and averaged across the ROI (Supplementary Fig. S1). This was then used to measure the change in the apparent R_2^* by modeling the average signal as $A(t) = A_0(t)e^{-TE \cdot R_2^*(t)}$. The source of the BOLD response is the dependence of R_2^* on blood oxygenation, but the problem that usually confounds the interpretation of slow modulations of the BOLD response is that A_0 is sensitive to scanner drifts. The direct calculation of R_2^* minimizes this source of error. For display and analysis, we calculated an equivalent δ BOLD response with A_0 removed as in Perthen et al. (Perthen et al., 2008) using the definition $\delta\text{BOLD}(t) = e^{-TE_2 \cdot \Delta R_2^*(t)} - 1$ where δBOLD is the percent change in the BOLD signal. Surround subtraction produces a net signal that is proportional to the arterial spins delivered to the voxel (Liu and Wong, 2005), and quantification of this signal in absolute CBF units was performed as described in Perthen et al. (Chalela et al., 2000; Perthen et al., 2008).

To calculate CMRO₂ from normalized CBF and BOLD data, the Davis model (Davis et al.,

1998) was used: $\delta S = M \left[1 - f^\alpha \left(\frac{r}{f} \right)^\beta \right]$. This model describes the BOLD response as a function of the normalized (activation/baseline) values of CBF (f) and CMRO₂ (r). Hypercapnic calibration was performed as described in Perthen et al. (Perthen et al., 2008) to determine the scaling parameter, M , which is the maximum BOLD response associated with the calibrated BOLD Davis model equation (Davis et al., 1998). This scaling parameter was then used to determine normalized CMRO₂ from which we calculated δ CMRO₂. The central assumption of this calculation is that mild hypercapnia does not alter CMRO₂, so that M can be calculated from the model using the measured CBF and BOLD responses to hypercapnia (Chen and Pike, 2010; Jones et al., 2005; Sicard and Duong, 2005). To test whether bias in M would affect our conclusions, we also analyzed our data with $M \pm 30\%$.

In addition to determining M , values for the parameters α and β must be assumed. The conventional values of these exponential parameters are $\alpha=0.38$ and $\beta=1.5$ based on the original development by Davis and colleagues (1998). However, we recently revisited the question of the accuracy of the Davis model with a more detailed four compartment model of the BOLD response that includes effects left out of the original derivation, including omission of the intravascular signal compartment and volume exchange effects as CBV changes, as well as the assumption that CBV changes are uniformly distributed across vascular compartments (Griffeth and Buxton, 2011).

With this analysis we found that the mathematical form of the Davis model provides a good fit to the BOLD response predicted by the detailed model as a function of changes in CBF and CMRO₂ if α and β are allowed to vary from their conventional values. We found best-fit values of $\alpha=0.14$ and $\beta=0.91$ using non-linear parameter optimization of the Davis model to simulated data produced by the detailed BOLD model. These values of α and β may seem counterintuitive when viewed in the context of the original derivation of the Davis model, but one should keep in mind that these values represent the best fit to a more complex model of the BOLD response that includes effects the Davis model was not originally intended to describe. Therefore the original biophysical meaning of these parameters should not be imposed on the new optimized parameters. To test whether the assumption of these parameter values affects the results of the study, we also analyzed our data with $\alpha=0.38$ and $\beta=1.5$.

All the BOLD, CBF, and CMRO₂ responses were expressed as a percent change from the pre-caffeine baseline and are denoted as δ BOLD, δ CBF, and δ CMRO₂. This allowed us to measure changes in the baseline state and also to compare in an absolute fashion the evoked responses pre- and post-caffeine. In addition, for comparison with previous studies where this was not possible we also expressed each response as a fraction of the immediately preceding baseline value.

3. Results

Table 1 shows the mean CBF, R_2^* and BOLD responses to 5% CO₂ inhalation along with the calculated M values for the two sets of Davis model parameters. The fractional BOLD and CBF time courses for hypercapnia are provided in the Supplementary Data (Fig. S2).

Using the dual-echo ASL sequence, surround average and difference data were determined for each TE from the alternating tag and control images and averaged over the region of interest (ROI) as shown for one subject in Figure 2. To show applicability of this technique to single subjects, curves for absolute CBF and R_2^* are shown for an one subject in Figure 3.

Group averages over the baseline and stimulus time points are included in Table 2. Using the CBF ROI, baseline CBF was reduced (pre=82.6±4.4 ml/100 ml/min versus post=59.9±3.5 ml/100 ml/min, $p<0.001$) following caffeine administration. The absolute Δ CBF response to the visual stimulus was also reduced post-caffeine (pre=42.0±2.6 ml/100 ml/min versus post=33.7±2.2 ml/100 ml/min, $p=0.033$). Baseline R_2^* was increased post-caffeine (24.4±1.1 s⁻¹ versus 27.1±1.2 s⁻¹, $p<0.001$) while ΔR_2^* in response to the visual stimulus was not significantly affected by caffeine (Fig 4b, pre=-0.50±0.4 s⁻¹ versus post=-0.51±0.03 s⁻¹, $p=0.67$).

Figure 4 shows the effect of caffeine on the stimulus-evoked response relative to the pre-caffeine baseline in the top row and relative to the baseline immediately preceding the stimulus in the bottom row. All results are shown as percent change from their respective baselines. All numerical values reported here for the CMRO₂ change are steady state values calculated from the average values of CBF and BOLD on the plateau of the baseline and the response using the optimized steady-state Davis model of the BOLD effect. Without the ability to directly measure changes in the baseline BOLD and CBF, the conventional analysis as displayed on the bottom row does not reveal the full extent of physiological modulation by caffeine (Fig. 4d-f). In fact with the conventional analysis, the effects of caffeine on the evoked responses to the stimulus were modest, with no change in the BOLD response, an insignificant change of 9.3% ($p=0.38$) in the evoked CBF response, and a 34% ($p=0.01$) increase in the CMRO₂ response (Table 3).

In contrast, the full quantitative analysis (Fig. 4a-c) showed four large effects of caffeine: a 27% ($p<0.001$) decrease in baseline CBF, a 22% ($p=0.030$) increase in baseline CMRO₂, a 61% ($p<0.001$) increase in the absolute magnitude of the CMRO₂ response to the stimulus, and a 20% ($p=0.036$) decrease in the absolute magnitude of the CBF response (Table 3). The time courses illustrated in Figure 4 for CMRO₂ should be taken as approximate because they were calculated by assuming that the steady-state model can be applied dynamically, but this may not be accurate (Buxton et al., 2004).

The importance of the increase of the stimulus-evoked CMRO₂ response is that it was not matched by a corresponding increase of the CBF response. The coupling of CBF and CMRO₂ responses can be described by an empirical index n , defined as the dimensionless ratio of the fractional changes in CBF and CMRO₂. In this experiment, n significantly decreased with administration of caffeine (pre=1.96±0.06 versus post=1.60±0.03, $p<0.001$) in agreement with previous findings (Chen and Parrish, 2009b). Even though the changes in pre-caffeine and post-caffeine n appear small, they represent significant changes in underlying physiology. These decreases of n are evident even for individual subject data (Supplementary Data, Fig. S3).

To test whether the choice of ROI biased our results, we used an ROI determined from the intersection of voxels exhibiting CBF activation and BOLD activation (as determined from the second echo average data). This produced very similar results: a 27% ($p<0.001$) decrease in baseline CBF, a 10% ($p=0.23$) increase in baseline CMRO₂ that did not reach significance, a 52% ($p<0.001$) increase in the absolute magnitude of the CMRO₂ response to the stimulus, and a 23% ($p=0.042$) decrease in the absolute magnitude of the CBF response. In the combined BOLD/CBF ROI, the coupling parameters were systematically higher due to a systematically higher BOLD response and lower CMRO₂ response. However, caffeine ingestion still resulted in lower n (pre=2.32±0.08 versus post=1.78±0.05, $p<0.001$). Full results are given in the Supplementary Data.

We also examined whether the choice of α and β would affect the results by using the parameters from the original Davis model, $\alpha=0.38$ and $\beta=1.5$. This resulted in a systematic

decrease in calculated δCMRO_2 but did not change our main conclusion that caffeine decreases n by increasing the CMRO_2 response (54%, $p<0.001$) and decreasing the CBF responses to the visual stimulus. Baseline CMRO_2 also increased with a trend toward significance ($13.3\pm6.4\%$, $p=0.067$) (Supplementary Data, Table S4).

Since the assumption that hypercapnia does not change CMRO_2 has been challenged (Kliefarth et al., 1979; Xu et al., 2011; Zappe et al., 2008), we tested whether our conclusions depend on the exact value of M . We repeated the analysis assuming a fixed value for M of $\pm 30\%$ of the average M from the hypercapnia experiment. This range brackets the results from a number of similar studies (Ances et al., 2008; Chiarelli et al., 2007; Hoge et al., 1999; Liao and Liu, 2009; Mark et al., 2011; Stefanovic et al., 2006) after adjusting for TE, α and β . Even with these large variations in M , we found that caffeine still decreased n by increasing the CMRO_2 response to the stimulus (Table S5). Baseline CMRO_2 also increased post-caffeine for different values of M with $M=8.3\%$ resulting in a strong 42% ($p=0.006$) increase in baseline CMRO_2 while $M=15.4\%$ resulted in a trend toward increased CMRO_2 (Table S5).

4. Discussion

Fluctuations in oxygen metabolism may in fact provide a much more accurate reflection of neural activity than changes in the BOLD signal alone since aerobic metabolism of glucose is the primary metabolic fuel for energy production in the human brain. Recent research has shown that changes in CMRO_2 are expected to reflect the underlying energy requirement of evoked neural activity (Lin et al., 2010), which is primarily the energy cost of pumping ions against their gradient at neuronal synapses (Attwell and Iadecola, 2002; Attwell and Laughlin, 2001). The importance of accurately and non-invasively measuring CMRO_2 is emphasized by the many common psychiatric and neurological diseases demonstrating changes in CMRO_2 such as schizophrenia (Gur et al., 1987; Hoyer and Oesterreich, 1975), depression (Videbech et al., 2001), bipolar disorder (Brooks et al., 2006; Yatham and Maj, 2010), Alzheimer's (Ishii et al., 1996), chronic traumatic encephalopathy (Zauner et al., 2002), and epilepsy (Greene et al., 2003). Therefore by improving our ability to non-invasively determine CMRO_2 dynamics, we will also unlock the ability to expand our knowledge of these diseases and, potentially, our ability to treat them. The current study demonstrates that a dual-echo calibrated-BOLD approach provides the capability of quantitatively assessing physiological changes in the brain in response to a drug, including both baseline changes and alterations in the response to a standard stimulus.

While CBF is a physiological measurement of direct interest, R_2^* is primarily of interest as a means to estimate CMRO_2 changes using hypercapnic calibration and a mathematical model of the BOLD effect (Davis et al., 1998). Typically, CMRO_2 changes are calculated relative to the baseline immediately preceding the stimulus. After administration of a drug, this relative CMRO_2 response could change either with a shift in the baseline CMRO_2 or with modulation of the absolute evoked CMRO_2 response to the stimulus. A novel feature of our analysis is that we were able to refer all changes pre- and post-caffeine to the pre-caffeine baseline state, which is possible because CBF and R_2^* are both relatively robust absolute values that allow for increase inter-study reliability as they are not affected by slow signal changes due to scanner drift. In this way we were able to measure both changes in the baseline state and also the ratio of the absolute evoked CMRO_2 responses to the stimulus.

The primary new finding of this study was that the absolute evoked CMRO_2 response to the stimulus was $\sim 61\%$ larger post caffeine, while the absolute CBF response decreased by $\sim 20\%$. This divergence of CBF and CMRO_2 responses to the stimulus was also evident in the baseline shifts due to caffeine. Baseline CMRO_2 increased by $\sim 22\%$ due to caffeine,

consistent with previous studies in rats showing a 15% increase in cerebral glucose utilization after 10mg/kg caffeine administration (Nehlig et al., 1984). Also consistent with previous studies, we found a significant reduction in baseline CBF by ~27% (Cameron et al., 1990b; Field et al., 2003; Laurienti et al., 2003; Mathew and Wilson, 1985). With the more conventional analysis, taking each response as a fractional change from its own preceding baseline condition, this combination of changes led to a reduction in the coupling ratio, n , of CBF and CMRO₂ responses to the visual stimulus, consistent with the finding of Chen and Parrish (Chen and Parrish, 2009b).

Of interest is that the large physiological changes found in this study in both the baseline state and in the response to the stimulus were not reflected in the BOLD response because they have opposing effects. The post-caffeine drop in baseline CBF with a corresponding increase in CMRO₂ required increased extraction of oxygen and consequently a higher level of deoxyhemoglobin in this baseline state, which acted to increase the magnitude of the evoked BOLD response post-caffeine. On the other hand, the CMRO₂ change evoked by the stimulus was much larger after caffeine administration while the CBF change was slightly smaller, and this reduced the magnitude of the BOLD response.

Previous results for the effect of caffeine on the evoked BOLD and CBF responses are mixed. For example, Chen and Parrish (2009a) found an increase in the fractional CBF response post-caffeine, and many groups have found the mean BOLD response to be increased (Behzadi and Liu, 2006; Chen and Parrish, 2009a; Morton et al., 2002; Mulderink et al., 2002) while others have found the peak BOLD response to be unchanged (Liu et al., 2004) or highly variable between subjects (Laurienti et al., 2003). Differences from the results of Chen and Parrish (2009a) are of particular interest due to the similarity of their approach. Both studies found that caffeine reduced the ratio of blood flow to oxygen metabolism changes in response to a visual stimulus, but the major difference in the current approach is use of the dual-echo acquisition, which allows for absolute quantification of R_2^* and CBF rather than only relative quantification. This is most apparent in Figure 4: the relative approach only allows for production of panels d–f while the dual-echo technique allows production of panels a–c. The latter panels also show the baseline shifts and permit comparison of the magnitudes of the absolute BOLD, CBF and CMRO₂ responses. The importance of this is emphasized when comparing the CBF results in Fig. 4b and 4e. Examining Fig. 4e, there is a small although insignificant increase in the relative response of CBF to the stimulus post-caffeine ($9.3 \pm 10.2\%$, $p=0.38$) similar to the results from Chen and Parrish (2009a). Yet when referred to the same baseline as in Fig. 4b, this insignificant increase becomes a significant decrease in the absolute CBF response.

Apparent inconsistencies in the literature regarding the effects of caffeine on the BOLD response might be explained by the current findings of opposing effects due to increased baseline deoxyhemoglobin combined with a decreased coupling ratio n post-caffeine. Depending on the particular set of subjects chosen and experimental conditions, it is easy to imagine this balance shifting to produce variable changes in the BOLD response. This potentially variable balance of opposing effects may also underlie the differential dose dependence of the CBF and BOLD responses for different levels of caffeine administration found by Chen and Parrish (Chen and Parrish, 2009a).

Our study involved subjects who were all moderate daily caffeine consumers who had not consumed any caffeine for at least 12 hours before the study. For this reason, it is more accurate to view this group as recovering from caffeine withdrawal during imaging. The relatively high baseline CBF pre-caffeine in these subjects (Table 2) suggests that adenosine or adenosine receptor levels had adjusted to the subjects regular levels of caffeine consumption. Abstaining from caffeine increased activation of the adenosine system in these

subjects elevating baseline CBF and suppressing baseline CMRO₂. With typical daily caffeine restored, baseline CBF was reduced to a level consistent with non-caffeine consuming controls (Rack-Gomer et al., 2009) while CMRO₂ was increased, also presumably to a more typical level. A similar experiment to the current one with caffeine-naïve subjects could yield significantly different results, and is an important area for future work.

A potential limitation of the current work is that the derived estimates of CMRO₂ response depend on the accuracy of the Davis model (Davis et al., 1998) for the relationship between the BOLD response and changes in CBF, cerebral blood volume (CBV) and CMRO₂. Potential limitations include the following possibilities: that the model is oversimplified, leaving out potential contributions to the BOLD signal; that the model does not adequately capture the effects of CBV change, particularly if CBV changes are primarily arterial; and that the hypercapnia calibration experiment may decrease baseline CMRO₂, contrary to the assumption of the calibrated-BOLD method (Buxton, 2010). In a recent modeling study we considered each of these limitations in detail (Supplementary Data, Table S4), and developed a detailed mathematical model of the BOLD signal against which we compared the Davis model (Griffeth and Buxton, 2011). Our primary finding was that despite the limitations of the original Davis model the mathematical form works well as a description of how the BOLD response varies as CBF and CMRO₂ are changed. However, because this simple form now describes effects that were not included in the original derivation of the Davis model, such as intravascular signal changes, the parameters should be treated as simply fitting parameters without any specific physical meaning. We used these optimized parameters for the primary estimates of CMRO₂ change reported here. To test whether these assumptions strongly affected the conclusions, we reanalyzed the data with the conventional Davis model parameters and found similar results (Supplementary Data, Table S4). It is notable that while the factors listed above modify the absolute values of the estimated CMRO₂ changes, the primary conclusion that caffeine increases the stimulus-evoked change in CMRO₂ is not changed. However due to the non-linear nature of the Davis model, the optimized parameters have a larger effect on calculations of CMRO₂ changes due to caffeine alone. While the classic Davis model parameters produce a trend for increased post-caffeine baseline CMRO₂, using the optimized parameters the increase was significant.

These small uncertainties in the exact values for CMRO₂ are less important than the broader implications that this study has for fMRI based on the BOLD effect. In this study, the BOLD response was insensitive to the physiological effects of an administered drug despite large changes in both the baseline state and the stimulus-evoked metabolic response. Combined with previous studies finding strong variation of the BOLD response with the baseline state (Brown et al., 2003) or with the CBF/CMRO₂ coupling ratio (Ances et al., 2008; Lin et al., 2008), these data support the general conclusion that the BOLD response should be interpreted with caution as a quantitative reflection of the underlying physiological changes. Our results also demonstrate that the quantitative approach used here, measuring both baseline and evoked response changes, can resolve many of the ambiguities of the BOLD response alone. The key element that makes this possible is the measurement of absolute CBF and R_2^* (Fig. 3 and Table 2). This approach will be useful for evaluating drug effects, and also for studies in disease populations where the baseline state may be altered due to the disease process itself or to medications.

Our results also have implications for our basic understanding of the connections between neural activity, blood flow and energy metabolism. The increase in the absolute magnitude of stimulus-evoked change in CMRO₂ suggests an increase in the overall evoked neural response, and yet this increase was not fully reflected in the CBF response. One possible explanation is that changes in CBF are not directly tied to CMRO₂ changes. Instead, CBF is

driven in a feed-forward manner by agents released by neural activity (Attwell and Iadecola, 2002; Hamel, 2006) or through activation of astrocytes (Iadecola and Nedergaard, 2007; Koehler et al., 2009). In contrast, CMRO₂ adjusts as needed to meet the energy requirements of the evoked neural response.

This picture of CBF and CMRO₂ driven in parallel, potentially by different aspects of neural activity, opens the theoretical possibility that the balance of CBF and CMRO₂ changes may vary depending on specific aspects of the neural activity change (e.g. input activity vs. evoked response, bottom-up vs. top-down modulation, etc). Our current results provide an example of this variability in the coupling of CBF and CMRO₂ revealing that the CMRO₂ response to the visual stimulus is increased post-caffeine, which is consistent with caffeine increasing neuronal excitability. The failure of CBF to respond as strongly post-caffeine might be due to the inhibition of the vasodilatory effects of adenosine by caffeine. Another possibility is that CBF is more strongly driven by the initial input stage of the neural response rather than the full ongoing evoked response. This is also consistent with the idea of feed-forward neurovascular coupling. Further experiments will be needed to evaluate these possibilities.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ASL	arterial spin labeling
BOLD	blood oxygenation level dependent
CBF	cerebral blood flow
CMRO₂	cerebral metabolic rate of oxygen
CBV	cerebral blood volume
fMRI	functional magnetic resonance imaging
R₂[*]	signal decay rate
TE	echo time

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Research Highlights

- Caffeine reduced baseline blood flow and increased baseline oxygen metabolism
- Caffeine increased the evoked oxygen metabolism response to the visual stimulus
- Despite these large physiologic changes, the BOLD response was unchanged
- Dual measurement of blood flow and BOLD allows these differences to be dissected
- Quantitative fMRI has promising applications for disease and drug studies

Fig. 1.

Experimental design for acquisition of simultaneous BOLD and CBF data showing stimulus pattern with a 60 s baseline followed by 4 cycles of 20 s of stimulus/60 s of rest and a final 30 s of rest. Tag and control images are alternated each TR (2.5 s), during which two echoes are acquired ($TE_1=2.9$ ms and $TE_2=24$ ms). For each echo, the surround average and surround difference of the tag and control images are calculated as shown in order to produce average and difference time courses for each echo as shown in Supplementary Figure S1.

Fig. 2. CBF-based activation map for a single subject, showing correlation coefficients calculated using the general linear model. Regions of interest (ROIs) were determined by thresholding the activation maps as described in the text.

Fig. 3.

Single cycle CBF and R_2^* . Black bar shows stimulus period of 20 s. **a**, Absolute CBF was calculated from echo 1 and echo 2 difference data for a single subject. **b**, Absolute R_2^* was calculated from average echo 1 and echo 2 data for a single subject.

Fig. 4.

Fractional changes in BOLD, CBF and CMRO₂ relative to either the pre-caffeine baseline (a–c) or relative to the baseline immediately preceding the stimulus (d–f). Blue time courses are pre-caffeine. Red time courses are post-caffeine. Black bars indicate stimulus on period of 20 s. Error bars indicate $\pm$ s.e.m. At baseline, error is considered relative to 0. Evoked response s.e.m. is considered relative to mean baseline shown. *Percent changes from the pre-caffeine baseline:* **a**, The BOLD baseline was shifted post-caffeine (change of $-6.27 \pm 1.1\%$, $p < 0.001$), while the BOLD response to the visual stimulus was not significantly different (change of $-3.64 \pm 7.3\%$, $p = 0.63$). **b**, There was a large overall decrease in the baseline CBF ($-26.9 \pm 3.5\%$, $p < 0.001$) and in the fractional δ CBF response post-caffeine ($-20.3 \pm 8.2\%$, $p = 0.036$). **c**, In contrast to the baseline CBF decrease, there was a trend for increased baseline CMRO₂ ($+21.8 \pm 8.4\%$, $p = 0.030$), and the δ CMRO₂ response to the visual stimulus was dramatically increased ($60.7 \pm 9.5\%$, $p < 0.001$) post-caffeine. *Conventional analysis showing percent changes relative to the baseline preceding the stimulus:* **d**, Baseline shifts are no longer apparent, and the BOLD response to the visual stimulus remained unchanged (pre = 1.20 ± 0.09 versus post = 1.23 ± 0.07 , $p = 0.67$). **e**, When considered relative to the baseline preceding the stimulus, the fractional δ CBF response increased slightly relative to the pre-caffeine response ($9.28 \pm 10.2\%$, $p = 0.38$). **f**, Similarly, the fractional δ CMRO₂ response also increased ($34.0 \pm 10.7\%$, $p = 0.01$) but less so than when considered relative to the pre-caffeine baseline.

Table 1**Response to hypercapnia**

Response to hypercapnia (standard error)

	CBF ROI
δ CBF (%)	29.5 (8.3) * $p=0.006$
ΔR_2^* (s ⁻¹)	-0.73 (0.14) **
δ BOLD (%)	1.78 (0.33) **
<i>M</i> , optimized (%)	11.9 (1.7) **
<i>M</i> , classic (%)	8.47 (1.2) **

Mean (one s.e.m.),

* $p<0.01$,** $p<0.001$, measured in the pre-dose caffeine hypercapnia experiment and calculated *M* values.

Note the *M* denoted as optimized refers to use of the new values of α and β and classic refers to use of the original values of α and β in the Davis model (Davis et al., 1998).

All significance values indicate a significant difference from zero.

Table 2Absolute CBF and R_2^* .

	CBF ROI	
	CBF (ml/100 ml/min)	R_2^* (s ⁻¹)
Pre-caffeine (baseline)	82.6 (4.4)	24.4 (1.1)
Pre-caffeine (stimulus)	124.6 (5.2)*	23.9 (1.1)*
Post-caffeine (baseline)	59.9 (3.5)*	27.1 (1.2)*
Post-caffeine (stimulus)	93.6 (4.8)*	26.6 (1.2)*

Mean (one s.e.m., *p<0.001), absolute values of CBF and R_2^* measured in the pre- and post-caffeine experiments (10 subjects). 'Baseline' is averaged over the 10 s prior to the start of the stimulus during which a gray background with a white square in the middle was presented. The stimulus response was averaged over the last 10 s of the stimulus and denotes the steady state response to the flashing checkerboard visual stimulus. Significance for the pre-caffeine stimulus response and the post-caffeine baseline shift were tested against the pre-caffeine baseline. Significance for the post-caffeine stimulus response was testing against the post-caffeine baseline.

Table 3
Fractional percent changes in CBF, BOLD, and CMRO₂

Fractional changes (standard error)

	δ CBF (%)	δ BOLD (%)	δ CMRO ₂ (%)	<i>n</i>
<u>Relative to pre-caffeine baseline (CBF ROI):</u>				
Pre-caffeine (stimulus)	52.1 (4.4) **	1.20 (0.09) **	26.7 (2.3) **	1.96 (0.06)
Post-caffeine (baseline)	-26.9 (3.5) **	-6.27 (1.1) **	21.8 (8.4) *, p=0.030	
Post-caffeine (stimulus)	41.5 (3.2) **	1.15 (0.06) **	42.9 (3.6) **	
Response percent change	-20.3 (8.2) *, p=0.036	-3.64 (7.3), p=0.63	60.7 (9.5) **	
<u>Conventional analysis relative to immediately preceding baseline (CBF ROI):</u>				
Post-caffeine (stimulus)	57.0 (3.6) **	1.23 (0.07) **	35.8 (2.3) **	1.60 (0.03) **
Response percent change	9.28 (10.2), p=0.38	2.96 (6.7), p=0.67	34.0 (10.7) *, p=0.01	

Nomenclature for baseline and stimulus are explained in the caption for Table 2. All significance values indicate a significant difference from zero except the values of *n* for which the post-caffeine *n* was compared to the pre-caffeine *n*. "Response percent change" refers to the percent change in the post-caffeine stimulus response compared to the pre-caffeine stimulus response ("a percent of a percent").

*
p<0.05,

**
p<0.001